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ABSTRACTS

Included in this booklet are the abstracts that will be presented at ISSFAL2012. This includes those that will be presented in Plenary Session, Symposia (both those invited and those selected from the submitted abstracts) or as Posters. Please note that the abstracts are listed with the presenting author first.

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Plenaries

Plenary Sessions

21st Century Preventive Cardiology: Lipoproteins not Lipids; Biomarkers, Anthropometrics and Meaningful Motivation; The Way to Our Patients' Hearts

Baum, Seth

Women's Preventive Cardiology; Boca Raton Regional Hospital

The number of deaths annually attributable to heart disease is diminishing, yet cardiovascular disease remains the largest killer in the western world. To make matters worse, obesity and concomitantly type 2 diabetes mellitus are on the rise. This fact portends a reversal in the last decade's positive trends in cardiovascular mortality. Unless we change our approach to cardiovascular prevention, the world will soon be faced with devastating health issues and their attendant financial ramifications. This lecture will focus on a more modern and effective approach to CV risk reduction. It will emphasize the superiority of lipoprotein over lipid analysis; the careful use of non-invasive imaging and biomarkers; and anthropometric evaluation as a means of risk reclassifying our patients. It will also include case presentations to depict the integration and efficacy of this model into every day clinical practice.

Why is it important to study the effects of dietary lipids on membranes?

Chapkin, Robert S.

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Two of the most abundant bioactive lipids enriched in fish oil, eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) are capable of altering cell membrane properties and resident protein activity. Recent evidence suggests that DHA can perturb specialized regions of the plasma membrane known as lipid rafts. Lipid rafts are mesoscale (2-300 nm), heterogeneous microdomains that are enriched in cholesterol, sphingolipids, polyphosphoinositides and saturated acyl chains. DHA is sterically incompatible with cholesterol, which can contribute to the disruption of lipid rafts. This is noteworthy, because lipid rafts serve as signaling platforms by compartmentalizing plasma membrane proteins and lipids. In response to stimuli, nanometer-scale domains can coalesce and display high molecular order. Many of these lipid raft mediated processes, e.g., epidermal growth factor receptor (EGFR) and Ras activation, play an integral role in driving tumorigenesis. Additionally, chronic inflammation, central to the process of tumorigenesis, involves excessive cell activation, which is in part regulated by lipid rafts. In addition, DHA, and to a lesser degree, EPA, can competitively remodel phospholipid molecular species containing arachidonic acid (AA, 20:4 n-6), e.g., ethanolamine/choline glycerophospholipids and polyphosphoinositides, thereby modulating eicosanoid biosynthesis and membrane cytoskeletal mediated cell signaling, respectively. Since AA-derived eicosanoids, e.g., PGE2, can regulate inflammation and promote cancer development, many investigators have targeted prostaglandin enzymes in an attempt to modulate AA metabolism. However, due to safety concerns surrounding the use of pharmaceutical agents designed to target Ptgs2 (cyclooxygenase II) and its downstream targets, it is important to identify new targets upstream of Ptgs2. Therefore, we determined the utility of antagonizing membrane AA levels as a novel approach to suppressing AA-derived eicosanoids. Overall, the knowledge obtained from mechanistic studies targeting cell membranes will provide a solid underpinning for the role of dietary lipids in the resolution of chronic inflammation and cancer prevention.

Membrane Lipid-Protein Function

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G protein-coupled membrane receptors (GPCR) transmit extracellular signals elicited by compounds like neural transmitters, hormones, odorants, or light to the cell interior where they activate GTP-binding

proteins (G proteins). This large superfamily of heptahelical molecules comprises receptors for dopamine, serotonin, epinephrine, opioids, and cannabinoids, just to mention a few. The proper function of GPCR is critical for all higher forms of life. We are conducting structural and functional studies on two reconstituted GPCR of class A, bovine rhodopsin and recombinant cannabinoid CB_2 receptor. The GPCR are investigated at close to functional conditions, in a fluid lipid matrix with a biologically relevant composition of lipids. Our studies place particular emphasis on polyunsaturated lipids as found in brain. The lipid matrix both preserves structural integrity of GPCR and enhances or prevents transition into the state of the receptor that activates G protein. I will report on adjustments of the lipid matrix to the presence of the receptor and on the influence of receptor function from lipid headgroups and hydrocarbon chains. The composition of the lipid matrix is, perhaps, the most important allosteric modulator of GPCR function. I will also address opportunities to study receptor structure in the lipid matrix at close to functional conditions by solid state NMR. Medical Director,

Genetics and fatty acid-binding proteins

Glatz, Jan F.C.

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Transport of fatty acids is regulated by membrane fatty acid transporters, e.g. CD36 and the FATPs, and by cytoplasmic fatty acid-binding proteins (FABPs). As a result, these proteins are implicated in various diseases that involve alterations in fatty acid transport and/or metabolism such as atherosclerosis, type 2 diabetes, and some neuropsychiatric and neurodegenerative diseases. In recent years common genetic variants of these proteins have been identified and were found to associate with circulating lipid profiles and metabolic phenotypes. For instance, subjects with CD36 gene variants that result in a lower CD36 expression level were found to be less susceptible to the metabolic complications of obesity. Likewise, common variants of the liver-type FABP were reported to influence insulin resistance and type 2 diabetes. In view of the still emerging functions of these fatty acid-binding proteins, such as the role of CD36 in fat taste perception and that of CD36 and cytoplasmic FABPs in lipid signal transduction and inflammation, common gene variants are expected to contribute to individual variability in these parameters. These new developments as well as our current understanding of the underlying molecular mechanisms will be discussed in this lecture.

Pathways for the Generation of Dysfunctional HDL

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The accumulation of cholesterol by artery wall macrophages plays a critical role in atherosclerosis, the leading cause of cardiovascular disease. HDL retards this process by promoting cholesterol efflux from macrophages by the ABCA1 pathway. HDL has been proposed to become dysfunctional in subjects with atherosclerosis, but the underlying mechanisms are poorly understood. One potential pathway involves myeloperoxidase (MPO), a potent source of reactive intermediates in human artery wall macrophages. We used mass spectrometry to demonstrate that apoA-I of HDL isolated from patients established heart disease exhibits site-specific chlorination of tyrosine, a characteristic product of MPO. When apolipoprotein A-I (apoA-I), the major HDL protein, was chlorinated by MPO, its ability to promote cellular cholesterol efflux by ABCA1 was impaired. Moreover, MPO-oxidized apoA-I was unable to activate lecithin:cholesterol acyltransferase (LCAT), which rapidly converts free cholesterol to cholesteryl ester, a critical step in HDL maturation. Biochemical studies implicated tyrosine chlorination and methionine oxygenation in the loss of ABCA1 and LCAT activity by MPO-oxidized apoA-I. Oxidation of specific residues in apoA-I inhibited two key steps in HDL maturation, raising the possibility that MPO initiates a pathway for generating dysfunctional HDL in humans.

Cellular and Circuit Level Imaging During and After Stroke

Murphy, Timothy H.

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During stroke, neurons that are deprived of their normal substrates can show signs of structural damage to dendrites after as little as 2 min of ischemia. Mitochondria also become depolarized within minutes after ishcemia. Mitochondrial dysfunction and the opening of the mitochondrial permeability transition pore (mPTP) are proposed to link ischemic ionic imbalance to mitochondrially mediated cell death pathways. Some neurons escape damage within the penumbra. Over time (weeks), surviving brain tissue is thought to compensate for regions lost to stroke. It is generally assumed that recovery is a process that occurs over weeks and involves both the formation of new structural circuits and the alternative use of spared circuits. Recovery after a small stroke may involve spared peri-infarct tissue with function similar to the infarct. In contrast, after a large stroke, tissue with similar function may only be found at more distant sites or regions within the unaffected contralateral hemisphere where structural remodeling can be observed. Using a large bilateral craniotomy preparation in mouse, we show that targeted ischemia to even a single arteriole causes alterations in the patterns of sensory-evoked activity that extend beyond peri-infarct areas into somatotopic regions of the unaffected hemisphere as early as 30 min after stroke onset. These findings suggest that existing sensory pathways are capable of redistributing activity to even the contralateral hemisphere. To assess changes in functional connectivity after stroke, we are developing an automated approach to monitor intrahemispheric and interhemispheric functional relationships by the activation of Channelrhodopsin-2 (ChR2)-expressing cortical neurons at arbitrary cortical points in transgenic mice. To monitor regional cortical activity we employ organic voltage sensitive dyes. We extend the point stimulation to areas targeting association cortices and secondary somatosensory regions that are inaccessible to direct stimulation via the senses and could potentially contribute to reorganized circuitry. We apply graph theory and complex network analysis to connection matrices derived from these functional maps to elucidate reciprocal connections between primary and secondary sensory areas, identify network hubs, and determine asymmetries in intracortical connectivity. We anticipate that new approaches to both monitor and manipulate neuronal function will be important to describe how spared cortical circuits compensate for brain tissue lost to stroke.

Fatty Acids and Regulation of Gene Expression

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Dietary fatty acids or fatty acids synthesized de novo in conjunction with nuclear receptors and transcription factors affect the transcription of a variety of genes. Several of these transcription mediators include the nuclear receptors peroxisome proliferator-activated receptor (PPARs), hepatocyte nuclear factor (HNF)-4 alpha, and liver X receptor (LXR) and the transcription factors sterol-regulatory element binding protein (SREBP), carbohydrate response element binding protein (ChREBP) and nuclear factor-kappaB (NFk-B). The mechanisms by which these interactions and consequent effects of the individual class of fatty acids occur is proving to be complicated and yet it is invaluable to our understanding of the role that dietary fat can play in disease management and prevention. We have used the stearoyl-CoA desaturase (SCD) mouse model to investigate the role of de novo synthesized fatty acids in the regulation of lipogenic gene expression. SCD catalyzes the de novo synthesis of monounsaturated fatty acids (MUFA) from saturated fatty acids. Past work demonstrated that SCD1 deficiency impairs hepatic lipogenesis and protects against diet-induced obesity. Our objective was to determine if hepatic MUFA synthesis is sufficient to restore the impaired lipogenic program in SCD1 global knockout mice (GKO). To address this, we produced liver-specific transgenic mice expressing human SCD5, which preferentially synthesizes oleate (18:1n-9), and introduced this transgene into GKO mice. Hepatic oleate synthesis increased plasma glucose levels and largely prevented very-low-fat diet-induced weight loss. Hepatic SREBP-1 maturation and lipogenic gene expression increased in hSCD5/GKO. This work suggests that hepatic MUFA are involved in regulation of lipogenesis and gluconeogenesis. Supported by NIH.

Nutrition, lipids and global child health

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The issue of lipid supply, in both its quantitative and qualitative dimensions, lies at the centre of some rapidly evolving changes affecting global nutrition patterns. Within a single generation populations that once were the focus of remedial supplementation programmes aimed at preventing malnutrition are now the focus of anti-obesity campaigns. Many countries have passed through the 'nutrition transition' and others are rapidly progressing. The desirability, affordability and abundance of refined vegetable oils has resulted in major changes in fat intake as a proportion of energy. The quality and type of oils is highly variable and may be having profound health effects that have yet to be properly explored. Despite this progress in many nations there remain large swathes of poverty where fat intakes may be marginal; creating a low-energy density in diets that compromises children's ability to ingest sufficient energy and potentially impairing the absorption of fat-soluble vitamins. Essential fatty acid intakes are frequently compromised in pregnancy and young childhood, though there is great heterogeneity among studies. Some of this heterogeneity may be caused by methodological issues, particularly in respect of accurately assaying the minor components of fatty acid profiles such as the long-chain PUFAs, and international ecological studies using standardised methodologies would be highly desirable. Lipid-based nutrient supplements (LNS) provide a particularly suitable vehicle for enhancing the essential fatty acid itakes of the most at-risk mothers and children and the many on-going research challenges will be summarised.

The Role of Highly Unsaturated Fatty Acids in the Health and Disease of the Retina

SanGiovanni, John Paul

NEI,National Institutes of Health, USA

The presentation will include: 1) an overview of extant work characterizing the capacity of diet-based fatty acids, their precursors, and metabolites to alter retinal structure and function; 2) discussion of a genomic systems-based approach to investigate relationship of receptors, transporters, enzymes and hormones impacting or impacted by these molecules in the context of pathogenesis of eye diseases manifesting neovascular and neurodegenerative components; and, 3) commentary on promising venues for development of preventive and therapeutic applications identified via the systems-based approach.

Novel PUFA-derived Mediators and Functional

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New endogenous mechanisms involved in the resolution of acute self-limited inflammatory exudates have provided insight into the control of both host defense and local acute inflammation. Using a systems approach coupled with lipid mediator (LM)-metabololipidomics that we introduced, permitted the identification of several new families of potent local acting bioactive lipid-derived mediators in resolving exudates (CN Serhan et al Nature Immunology 2008). This presentation shall update new advances on the biosynthesis and functions of the founding members of this novel genus of specialized pro-resolving mediators (SPM) and their roles as agonists of resolution. The SPM include 3 families of chemical mediators: resolvins, protectins and the most recent addition, maresins from macrophages. These are local autacoids biosynthesized in resolving exudates from essential omega-3 fatty acids (n-3, EPA and DHA) that possess potent multi-pronged anti-inflammatory, pro-resolving, reduce pain and microbial clearance actions in animal models. Low dose aspirin also triggers production of endogenous chiral epimers from certain of SPM pathways that have to be proven bioactive and stimulate resolution. Many other research groups worldwide now confirm endogenous formation of resolvins and protectins and their organ-protective roles and the first Rv is currently in human clinical trial. For example, SPM have potent actions in murine ischemic renal injury, obesity-induced insulin resistance and liver disease, murine colitis and arthritis, as well as reducing pain. New results from the author's research laboratory indicate that resolvins regulate specific microRNAs in a receptor dependent fashion that play key roles in active resolution. Identification of endogenous SPM biosynthesized locally and temporally during acute

inflammatory responses indicates that the resolution of acute inflammation is an *active programmed process* that also stimulates tissue regeneration. These findings change the old concept that resolution of inflammation is a passive process. Together, they indicate that natural resolution pathways may underlie many prevalent diseases associated with uncontrolled inflammation and open the potential for resolution-based therapeutics.

Triglyceride Digestion and Transport

Tso, Patrick

The focus of my talk will be on the digestion and absorption of dietary biliary lipids in the gastrointestinal tract, and the physiology of this event. The digestion of dietary triacylglycerol (TG) begins in the stomach and continues in the intestinal lumen. Both gastric lipase and pancreatic lipase contribute to its digestion. Following this initial breakdown of dietary TG, the hydrolytic products, monoacylglycerols (MG) and fatty acids (FA), are solubilized in micelles and taken into the enterocyte by both passive as well as carrier mediated processes. The importance of the unstirred water layer to the uptake of TG digestion products, as proposed by Dr. John Dietschy, will be described. I will also focus on the uptake of cholesterol by the enterocytes, via the NPC1L1 transporter. Following uptake into the enterocute, MG and FA are transported from the apical membrane to the endoplasmic reticulum, and re-esterified to form TG involving various enzymes. I will also focus on the mechanism of chylomicron formation in the enterocytes, and the role of the microsomal triglyceride transfer protein in regulating the process of chylomicron formation. Additionally, the trafficking of chylomicron particles from the endoplasmic reticulum to the Golgi apparatus will be described, as well as the process of chylomicron exocytosis and how they journey to the lacteals. I plan to conclude the talk with a discussion of the role of various apolipoproteins in intestinal lipid transport by the enterocytes and how the conscious lymph fistula mouse model has contributed to our knowledge of the subject.

Fatty Acids and the Immune System: Manifestation, Models and Mechanisms

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Since the first papers described the modulation of immune function by fatty acids in the 1970s, there have been significant developments which have explored the manifestation of these effects, used a wide range of models to evaluate different biological and clinical settings, and investigated underlying mechanisms. This lecture will examine the relationship between fatty acid composition and immune function, evaluate the influence of ageing on responsiveness of the immune system to n-3 polyunsaturated fatty acids (PUFA), describe the role of lipid rafts in immunomodulation by fatty acids, highlight advances in eicosanoid biology, and describe emerging receptors as targets for action by fatty acids.

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Symposia Presenters

Symposia Talks

Docosahexaenoic acid (DHA) deficiency impairs fusion protein organization and ultrastructural morphology in mouse spermatids

Abbott, Timothy L.; Manuel Roqueta-Rivera, Mayandi Sivaguru, Rex A. Hess, Manabu T. Nakamura

Division of Nutritional Sciences, Department of Food Science & Human Nutrition, Institute for Genomic Biology, Department of Comparative Biosciences, all of the University of Illinois at Urbana-Champaign

Spermiogenesis is the process whereby post-meiotic round spermatids are transformed into elongated spermatids. Among the most critical developments in this process is the biogenesis of an organelle unique to sperm, the acrosome, whose construction is dependent on stage-specific vesicular trafficking and membrane fusion events. Deficiency in docosahexaenoic acid (DHA) was recently shown to result in a failure of acrosome biogenesis; however a role for DHA in membrane fusion has yet to be defined. Here, we use Fads2-/- mice to investigate the effect of a DHA deficiency on intracellular trafficking and membrane fusion in spermiogenesis, in vivo. We show, using electron microscopy, that at the Golgi phase of spermiogenesis proacrosomal vesicles of Fads2-/- spermatids are successfully released from the trans-face of the Golgi apparatus but fail to coalesce to form the larger proacrosomal granules characteristic of late Golgi-phase spermatids. Similarly, we show by immunohistochemistry that the intracellular localization of acrosin (a cargo protein of proacrosomal vesicles) is normally distributed in early Golgi-phase spermatids, but in subsequent phases of spermiogenesis is dispersed throughout the cytosol in an abnormal punctate pattern. Further, membrane fusion proteins syntaxin2 and VAMP4 displayed aberrant accumulation throughout spermiogenesis; and endoplasmic reticulum cisternae, as well as smaller transport vesicles, were present in excess on the cis-face of the Golgi, each suggestive of impaired intracellular transport or fusion. In conclusion, acrosome biogenesis under DHA deficiency is halted after the release of proacrosomal vesicles from the Golgi; the mislocalization of syntaxin2, VAMP4 and acrosin in Fads2-/- spermatids suggests a possible role for DHA in certain specialized systems of intracellular trafficking and membrane fusion.

Potential use of dietary ω-6/ω-3 fatty acid ratios as chemopreventive tools against colon cancer development

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Understanding the differences in the lipid and fatty acid (FA) profiles between cancer and normal tissue in the colon, and the contribution dietary fat intake plays, may be invaluable in clarifying their role in carcinogenesis. With high dietary ω-6 polyunsaturated fatty acids (PUFA) intakes being linked to carcinogenesis, increased use of dietary ω-3 PUFA and resultant decrease in dietary ω-6/ω-3 FA ratio have been proposed to reduce cancer risk.

This investigation was a 3 stage study: (1) To compare the lipid profile of azoxymethane (AOM) induced colon polyps to that of surrounding mucosa tissue in rats fed a sunflower oil diet with a high ω-6/ω-3 FA ratio, (2) To evaluate the modulating effect of diets with specific ω 6/ω 3 FA ratios obtained from different dietary oil combinations on lipid membrane parameters and oxidative status in normal rat mucosa, and (3) To determine whether these specific FA ratios modulate aberrant crypt foci (ACF) development.

Colon polyps demonstrated a significantly modified lipid profile associated with the increased ω-6 PUFA content, likely enhancing polyp growth. Modulation of normal rat colon mucosa highlighted the differential effects of fish and borage oils on certain lipid and FA parameters. In comparison to the high ω 6/ω 3 ratio sunflower oil diet, fish and borage oil containing diets reduced the mucosa ω 6/ω 3 ratio. Oxidative status monitored by measuring lipid peroxidation (LPO), was significantly increased with the low ω 6/ω 3 FA ratio diets. ACF formation was significantly enhanced by fish oil, whereas borage oil counteracted this effect.

Modulation of the colon mucosa appears to be deferentially influenced by the type of fat constituting the ω-6/ω-3 FA ratio diet. These changes may potentially prime cellular conditions for cancer cell elimination

through oxidative stress related mechanisms. However, the efficacy of the intervention appears to be determined by the respective stage in carcinogenesis.

Retinal Very Long Chain Polyunsaturated Fatty Acids: There is More to Them than We Know

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Background: Retinal photoreceptors, testis, sperm, skin, and brain are uniquely enriched in glycerophospholipids and sphingolipids containing very long chain polyunsaturated (VLC-PUFA) and/or saturated (VLC-FA) fatty acids. These C28-C40 fatty acids are synthesized via Elongation of Very Long Chain Fatty acids-4 (ELOVL4) protein. Mutations in the ELOVL4 gene cause autosomal dominant Stargardt-like macular dystrophy (STGD3), intellectual disability, spastic quadriplegia, and ichthyosis in humans. Homozygote Elovl4 knockout (KO) and knock-in (KI) mice lack skin VLC-FA-containing sphingolipids and die after birth due to defects in skin barrier permeability while the heterozygote KI and KO mice have diminished retinal phosphatidylcholine-containing VLC-PUFA, and develop progressive retinal degeneration.

Objectives: We hypothesized that reduced/absence of VLC-PUFA contributes to the retinal and neurological pathology seen in STGD-3 patients and have designed experimental approaches to elucidate the role of these fatty acids in retina and brain development.

Procedures: In order to study the effect of complete absence of VLC-PUFA on brain and retinal function,we developed analytical methods for VLC-PUFA/VLC-FA glycerophospholipid and sphingolipid quantification. We used a skin-specific promoter to drive the expression of ELOVL4 protein thereby generating animals with global Elolvl4 deletion except in the skin. Conditional retinal mutant Elovl4 expressing mice were also generated by crossing CHX10-Cre-Elovl4-floxed mice with KI mice and used electroretinography (ERG), histology, and VLC-PUFA/VLC-FA quantification to study the effects of total loss of ELOVL4 protein and hence VLC-PUFA on retinal function and structure.

Results: Conditional expression of mutant Elovl4 in the retina produced mice (CHX10-Cre+ Elovl4-f/mutant) that are healthy but have reduced retinal function as measured by ERG at 4 months of age. Consequently, absence/reduction of VLC-PUFA/VLC-FA severely affects normal mouse development. The skin rescued mice are runts, have defects in eyelid opening, and die within the first three weeks from birth.

Conclusions: VLC-PUFA play uniquely important roles in normal development and neurological function.

Organelle Lipidomics Coupled to Proteomics: The Lipid-LOPIT approach

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Cells control their lipid composition very tightly as lipids have essential roles in signalling, growth, proliferation, apoptosis and other cell functions. This regulation extends to the sub-cellular compartments that make up the cellular architecture. Moreover, lipids help recruit proteins to specific locations and maintain their function. Determining spatiality at the subcellular level how the lipid composition varies is therefore very important in terms of understanding the role sub-cellular compartmentation plays in regulating cellular metabolism. Since lipids are known to reside in more than one specific organelle we

have expanded the well established proteomic approach of LOPIT (Localisation of Organelle Proteins by Isotope Tagging) to accommodate our lipidomic datasets.

To demonstrate this approach this study reports a comprehensive analysis of the subcellular proteome and lipidome of a hepatoma cell line (FaO) and its utility for monitoring sub-cellular changes associated with Non-Genotoxic Carcinogens (NGCs) exemplified by Mono(2-ethylhexyl) phthalate (MEHP).

A self-generating density gradient was used to partially separate organelles into individual fractions. Fractions were selected based on their profile of proteins, measured by western blotting. Distributions were then determined by isobaric mass tagging, LC-MS and multivariate data analysis, based on proteins with known organelle locations. This analysis of enriched organelle fractions identified numerous lipid species, whose type and concentration varied between different organelles. Following MEHP treatment, changes were seen in the observed distribution and concentration of the lipids including PtdEtn and PtdIns throughout the gradient, supporting the hypothesis that the some of the changes in the lipid profiles, due to NGCs, are a result of induced organelle proliferation.

The results represent the first use of Lipid-LOPIT in any cell line, the first application of LOPIT to a hepatoma cell line (FaO) and the first practical application of Lipid-LOPIT for determining early changes caused by a NGC.

Real time fatty acid profiling using ion mobility separation coupled to mass spectrometry

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The analysis of fatty acid composition often requires very laborious and time comsuming procedures. Here we present a rapid (few seconds), real time method to analyze the fatty acid profiles in food and biological samples. To achieve our goal we combined two emerging technologies - direct analysis in real time, DART (IonSense, US) and ion mobility spectrometry coupled with time-of-flight; Synapt G2 HDMS (Waters Corporation, UK). To illustrate the potential of this approach we analyzed lipid profiles of edible oils (e.g., fish oil and olive oil), lipid extracts from biological tissues and sebum, (oily matter that lubricates and waterproofs human skin). Samples were swiped on a capillary and placed near the mass spectrometer ion source. Lipids were ionizated by DART, separated by ion mobility (10-20 msec) and mass analyzed by the time-of-flight mass analyzer. This generated 3D maps (drift time, exact mass, and intensity) of the sample composition at the molecular level. Our approach of analysis is suitable for the rapid screening of various bioactive lipids, e.g., fatty acids and ceramides. Potential applications include fingerprinting of biological phenotypes and comparative lipidomic analysis in the areas of personalized medicine, disease diagnostics and food analysis.

Effect of conjugated linoleic acid supplementation on the human platelet proteome

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Cardiovascular disease is responsible for one third of the early deaths in the UK. Consumption of a diet high in saturated- and trans- fatty acids increases risk of cardiovascular disease. However, consumption of the dietary trans fatty acids conjugated linoleic acids (CLA) may protect against cardiovascular disease and improve platelet function. Many issues relating to the potential mechanism of protection are unknown.In a double-blind, randomized, placebo controlled parallel-group trial 40 overweight but healthy human adults supplemented their diet with 4 g CLA-enriched oil (80 % c9,t11-CLA, 20 % t10,c12-CLA) or placebo oil (80 % palm oil and 20 % soybean oil) per day for three months. The placebo oil mimicked the

fatty acid composition of an average Western diet. Platelet proteins from washed human platelets were separated in a total of 584 protein spots using 2-dimensional gel electrophoresis. Treatment with CLA, compared with placebo, significantly regulated levels of 46 valid platelet protein spots (p<0.05). Of these, 44 proteins were identified using LC-ESI-MS/MS. Pathway analysis based on KEGG pathways revealed that the majority of these proteins was involved in regulation of the cytoskeleton and platelet structure, or receptor activity, signalling and focal adhesion. The proteins CDC42, protein kinase Cδ, alpha-actinin-1 and integrin alpha-IIb precursor represented important protein hubs that were regulated by CLA. These proteins, or indeed downstream proteins or metabolites, could be candidate biomarkers to measure the efficacy of fatty acids on platelet function in future nutritional intervention studies.

Decrease of anandamide ratio between visceral and subcutaneous adipose tissues by dietary EPA and DHA phosphatidylcholine improves metabolic syndrome in obese zucker rats

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Endocannabinoid balance between visceral (VAT) and subcutaneous (SAT) adipose tissues may regulate lipid deposition and metabolism influencing several parameters of the metabolic syndrome. We have recently shown that dietary EPA and DHA incorporated into phospholipids, as in krill oil, is able to influence biosynthesis of the endocannabinoids in VAT but not in SAT.

In the present study we fed Zucker rats with 0.3% in the diet of EPA and DHA, corresponding to about 0.5%en and to humans 1.2g/d, either in the TAG (n-3TAG) or PC (n-3PC) forms for 4 weeks.

The results show that both dietary n-3PC and n-3TAG decreased significantly the endocannabinoid anandamide (AEA) in VAT but not in SAT. However, the ratio of AEA between VAT and SAT was significantly lower in rats fed n-3PC with respect to those fed n-3TAG. In addition, the decreased ratio was associated to a lower deposition of ectopic fat in liver and heart, plasma NEFA, glycemia and insulin resistance.

We may conclude that endocannabinoid balance between visceral and subcutaneous adipose tissues is crucial for body fat homeostasis and low doses of dietary n-3PC are able to affect significantly this balance and significantly improve metabolic syndrome in obese zucker rats.

What is the Role of Cytochrome P450 Epoxypoxygenase Metabolites of Arachidonic Acid in the Metabolic Syndrome?

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University of Western Australia, Australia

Background: Overweight people are at risk of developing heart disease due to their predisposition of also having high blood pressure, lipids and glucose levels. The cytochrome P-450 metabolites of arachidonic acid are important regulators of vascular function and homeostasis. This study examined metabolism of arachidonic acid by the cytochrome P450 epoxygenase that leads to formation of 4 epoxyeicosatrienoic acid (EET) regioisomers. EETs are vasodilators, and inhibit platelet aggregation. Their actions are attenuated by metabolism to dihydroxyeicosatrianoic acids (DHETs) by soluble epoxide hydrolase.Their possible contribution to cardiovascular risk has not been assessed in overweight humans.

Aim: To compare EETs and DHETs in plasma and platelets, in a case control study of untreated men and women with the metabolic syndrome (MetS).

Design: Plasma and platelet EETs and DHETs were measured by gas chromatography mass spectrometry in 16 cases and age and gender matched controls.

Results: The volunteers were aged 55.9±1.5y (MetS) and 54.5±1.5y (controls) with BP and BMI of 135/87±2.1/1.6mmHg and 34.3±1.2kg/m2 respectively, (MetS); and 112/69±2.1/1.6 mmHg and 24.2±1.2kg/m2, controls. Plasma EETs were increased in the MetS (7.7±0.39 ng/ml) compared with controls (6.22±0.35ng/ml), P=0.007. Plasma DHETs were not different between the groups 11.6±0.95 ng/ml (MetS) compared with 11.5±1.06 ng/ml (controls). In contrast to plasma, platelet EETs were

significantly reduced in the MetS (1.41±0.1ng/109cells) compared with controls (2.12±0.30 ng/109cells), P=0.04. Platelet DHETs were not different between the groups 0.39±0.04 ng/109cells (MetS) compared with controls, 0.46±0.06 ng/109cells.

Conclusions: The increase in plasma EETs in the MetS may be a homeostatic response to elevated blood pressure or increased circulating vasoconstrictors that have been linked to insulin resistance in these subjects. The reduced platelet EETs levels may be of relevance to increased platelet reactivity and aspirin resistance that has been described in subjects with the metabolic syndrome.

The Effects Of Infant Formula Beta-Palmitate Structural Position On Bone Speed Of Sound: A Double Blind, Randomized Control Trial

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Palmitic-acid presents 17-25% of human milk fatty acid content, with 70-75% in the sn-2 position of glycerol backbone (beta-palmitate) and is well absorbed. Contrary, palmitic-acid in the sn-1 and sn-3 positions, the predominantly fat composition in regular infant formulas, is hydrolyzed by pancreatic lipase, resulting in free palmitic-acid that forms poorly absorbed complexes with calcium, also associated with lower bones calcium deposition and abdominal discomfort.

The aim of the study was to compare the effect of 12 weeks feeding of infant formula with beta-palmitate (InFat™, produced by Advanced Lipids AB, JV of Enzymotec and AAK) versus regular formula on bone strength and crying.

Methods: Eighty-three term infants were enrolled; 58 formula-fed randomly assigned to formula with beta-palmitate (43% of the palmitic acid is esterified to the sn-2 position of glycerol backbone, InFat group, n=30), or regular formula (13% of the palmitic acid is esterified to sn-2 position of glycerol backbone, Control group, n=28) and 25 breastfed that served as reference group. Anthropometrics and bone SOS by quantitative ultrasound (Sunlight Omnisense) were measured at randomization, and at 6 and 12 weeks postnatal. Before each visit parents filled a three days report on infant feeding, stool characteristics and crying.

Results: There were no significant differences in anthropometrics at randomization and at 12 weeks postnatal. At 12 weeks postnatal mean bone SOS of the InFat group was significantly higher than that of the Control group (2896±133 vs 2825±79 m/ sec respectively, p=0.049), and comparable to the breastfed group (2875±85 m/sec). Infants in the InFat group had less episodes of crying per day and significant decrease in daily crying compared to Control group.

Conclusion: The consumption of InFat formula by term infants for 12 weeks had beneficial effects on bone SOS compared to regular Control formula and was most similar to the breastfed group.

The Differential Effects of EPA and DHA Omega-3 Fatty Acids on Brain Functioning

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Background: Recent evidence suggests that, although only present in low quantities in the brain, eicosapentaenoic acids omega-3 fatty acid (EPA), enhances early visual cortical processing measured with multifocal visual evoked potentials (Bauer et al., 2011 in print). However, while docosahexaenoic omega-3 fatty acid (DHA) has been shown to increase neural activity during a sustained visual attention task (McNamara et al.,2010) , the effect of EPA on neural mechanisms underlying higher order cognitive skills such as attention is still unknown.

Objective: To gain more insight into the neurocognitive effects of an EPA-rich supplementation (590 mg/d) compared to a DHA-rich formula (417 mg/d). Blood oxygen-level dependent functional magnetic resonance imaging (BOLD-fMRI) was used to determine neural changes during a Stroop Color Word Interference Task.

Design: Eleven healthy participants aged 20-34, with no known neurological or psychiatric disorders, not currently taking any nutritional supplementation, were recruited. Supplementations were administered using a double-blind, crossover design, with a 30-day washout period between the two supplementation periods. Participants were scanned at Baseline (prior to supplementation), and after each 30-day supplementation period. Reaction times and response accuracy were recorded.

Results: The EPA-rich supplementation was associated with a reduction in functional activation in the anterior cingulate cortex (ACC) when compared to Baseline. Further, reaction times were significantly reduced after EPA supplementation compared to DHA.

Conclusions: Reduced brain functional activation, coupled with faster reaction times during a Stroop Color Word Interference Task suggests a mechanism of improved neural efficiency following a 30-day EPA-rich supplementation.

Effects of iron and n-3 fatty acid supplementation, alone and in combination, on cognition: a randomized, double-blind, placebo controlled intervention in iron-deficient South African school children

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Background: Little is known about the combined effects of iron and n-3 fatty acid (n-3 FA) supplementation in children suffering from both deficiencies.

Objective: We investigated whether providing iron and a mixture of docosahexaenoic (DHA) and eicosapentaenoic acid (EPA), alone and in combination, to children with iron deficiency and poor n-3 FA intakes will improve cognitive performance.

Procedure: In a 2-by-2 factorial, double-blind trial, iron-deficient South African children (n=321, aged 6-10 y) were randomly allocated to receive 1) DHA/EPA (80/420mg) + iron (50mg as ferrous sulphate); 2) DHA/EPA + placebo; 3) placebo + iron; or 4) placebo + placebo as oral supplements (4 x per week for 8.5 months). Biochemical indicators and cognitive performance, using the Hopkins Verbal Learning Test (HVLT) and subscales of the Kaufman Assessment Battery for Children, were assessed at baseline and endpoint.

Results: Iron and n-3 FA status significantly improved with iron and DHA/EPA supplementation, respectively. A significant intervention effect of iron was found on HVLT Recall 2 (estimated effect size: 0.88, 95%CI: 0.17, 1.60), but there were no other significant effects on cognitive performance. However, separate analyses for subjects that were anaemic (n=64) and non-anaemic (n=227) at baseline revealed an effect of iron for increased scores in the Atlantis delayed (1.47, 95%CI: -0.04, 2.98), and an effect of DHA/EPA for decreased scores in the Atlantis test (-1.77, 95%CI: -3.28, -0.27) in the anaemic subjects. Iron supplementation significantly increased HVLT Recall 2 (2.02, 95%CI: 0.57, 3.48) and total HVLT Recall scores (3.01, 95%CI: -0.20, 6.32) in the anaemic subjects. No effects on cognition were found in the non-anaemic children.

Conclusions: Our findings suggest that iron supplementation may improve verbal and non-verbal learning and memory in children suffering from anaemia. In contrast, DHA/EPA supplementation had no effect on cognition and may even be detrimental in anaemic children.

DHA Homeostasis and Docosanoids Bioactivity in Experimental Stroke

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The significance of the selective enrichment in omega-3 essential fatty acids in the nervous system has remained, until recently, incompletely understood. While studying cell survival in neurodegenerations, we contributed to the discovery of a docosanoid synthesized from DHA by 15-lipoxygenase-1, which we dubbed neuroprotectin D1 (NPD1,10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15E,19Z hexaenoic acid). We found that NPD1 is promptly made in response to oxidative stress and brain ischemia-reperfusion, and in the presence of neurotrophins. Thus we envision NPD1 as a protective sentinel, one of the very

first defenses activated when cell homeostasis is threatened stroke or neurodegenerations. We will present the following studies: 1) DHA (i.v.) one hour after two hours of middle cerebral artery occlusion (MCAO) leads to penumbra protection with an entended time window (up to five hours) and with concomitant NPD1 synthesis. Neurological function was evaluated on up to 7 days after MCAo. DHA improved behavioral scores and reduced cortical , subcortical and total infarct volumes 7 days after stroke. In addition, DHA reduced microglia infiltration and increased number of astrocytes and neurons. 2) DHA activated p473 AKT and p308 AKT, and also increased pS6 and pGSK . DHA or NPD1 reduced total, cortical and subcortical infarct volumes in aged rats. Aged rats treated with DHA had increased NPD1 production after MCAo when compared to both young and aged rats treated with vehicle. The phosphorylation of p308 AKT or pGSK was not different between groups in aged rats. However, pS6 expression was increased with DHA or NPD1 . 3) A novel biosynthetic pathway that leads to the formation of AT-NPD1 in the brain will be presented. When we administered aspirin plus DHA we discovered COX-2 mediated synthesis of aspirin-triggered NPD1 (AT-NPD1). Then we performed the total chemical synthesis of this molecule and tested the novel AT-NPD1 by iv 1 hr after 2h middle cerebral artery occlusion in rats. On day 7, ex vivo MRI displayed T2WI, 3D volumes greatly reduced. Also reactive astrocytes, activated microglia/macrophages and SMI-71-positive vessels were reduced in the penumbra. Brain edema, computed from T2WI in the cortex (penumbra) and striatum (core), was elevated in the saline group.

In conclusion NPD1 targets neuroinflammatory signaling at various check points as well as modulates apoptotic cascades and other forms of cell death in turn promoting homeostatic regulation of neuronal circuitry integrity in experimental stroke. (Supported by NIH: NINDS R01 NS046741, NEI R01 EY005121)

Regulating brain PUFA concentrations: Uptake and rapid metabolism

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

A randomised placebo-controlled trial of ethyl-eicosapentaenoic acid and/or vitamins E + C in schizophrenia and related psychoses

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Background: Membrane phospholipid metabolism and redox regulation may be disturbed in schizophrenia. This has entailed randomised controlled trials with either omega-3 fatty acids or redox regulators. The present study examines the effects of combining these agents. We hypothesized that lower baseline levels of polyunsaturated fatty acids (PUFA) would predict more benefit from trial drugs.

Methods: This add-on clinical trial had a multicenter, randomized, double-blind, placebo-controlled, fixed dose, 2x2 factorial design. Patients aged 18-39 years with DSM-IV schizophrenia, schizoaffective or schizophreniform disorders were consecutively included at admission to hospital. Participants received active or placebo ethyl-eicosapentaenoate (EPA) 2 g/day and active or placebo vitamin E 364 mg/day + vitamin C 1000 mg/day (Vitamins) for 16 weeks. Effects on the Positive and Negative Syndrome Scale (PANSS), vital signs, biochemical variables and adverse events were analysed by linear mixed models.

Results: Ninety-nine patients were included. At baseline, erythrocyte PUFA were measured in 97 subjects. PUFA were bimodally distributed (low, high). Drop-out rates were three times higher among low than high PUFA patients (11/30 versus 8/67; P=0.007). In low PUFA patients, EPA alone impaired the course of total PANSS (Cohen's d=0.29;P=0.03) and psychotic symptoms (Cohen's d=0.40;P=0.003), whereas Vitamins alone impaired the course of psychotic symptoms (Cohen's d= 0.37; P=0.005). Adding Vitamins to EPA neutralized the detrimental effect on psychosis (Cohen's d= 0.31; P=0.02). In high PUFA patients, there were no significant effects of trial drugs on PANSS scales. Trial drugs had clinical effects beyond psychotic symptoms, mainly in low PUFA patients.

Conclusions: EPA and vitamins E+C given separately in moderately high doses can be harmful to patients with acute psychosis and low PUFA levels. When these agents are combined, they seem safe.

Omega-3 polyunsaturated fatty acids and their oxygenated derivatives increased adiponectin secretion

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Diets rich in long-chain w-3 polyunsaturated fatty acids, namely docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have been shown to have many beneficial effects such as the improvement of insulin sensitivity with beneficial effects against obesity and the prevention of cardiovascular diseases. The aims of this study were 1) to determine the effects of DHA and EPA supplementation on adiponectin secretion in mice fed a DHA- or an EPA-enriched-diet. The content and the expression of adiponectin in adipose tissues were also measured 2) to evaluate the effects of DHA and EPA and their respective oxygenated metabolites on adiponectin secretion by 3T3-L1 adipocytes.

Mice were fed either a control diet or a DHA- or an EPA-rich diet and were sacrificed on day 0 or 4. Additionally, 3T3-L1 cells were treated with either DHA, EPA or their oxygenated derivatives.

We show that DHA and EPA and their metabolites increased secreted adiponectin from 3T3-L1 adipocytes. Increased adiponectin secretion was also observed in plasma of mice as early as 4 days after initiation of the DHA- and the EPA-rich diet (+22% et +17%, respectively). In all three white adipose tissues (subcutaneous, epididymal and retroperitoneal tissues), the adipokine content was not significantly different between mice fed the w-3 polyunsaturated fatty acid-rich diet and mice fed with the standard diet. However, the adiponectin content was dependent on adipose tissue depot. Adiponectin gene expression was significantly increased in epididymal and subcutaneous tissues of DHA-fed mice compared to control mice.

Our studies show that DHA and EPA rapidly improved the profile of secreted adiponectin in mice and in 3T3-L1 adipocytes and suggest that this effect may be mediated by their respective oxygenated metabolites. These data confirm that dietary intake of w-3 polyunsaturated fatty acids may be beneficial for prevention or treatment of cardiovascular and obesity-associated diseases.

Myristoylated and palmitoylated proteins in the regulation of apoptosis and metabolism revealed through proteomic

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Fatty acylation, the attachment of fatty acids to proteins, is a critical mechanism of cellular control. It impacts virtually every aspect of cellular life. Progresses in the study of protein fatty acylation were hampered by the lack of rapid detection methods, which typically relied on the incorporation of radioactive

fatty acids into proteins followed by lengthy film exposures. Using chemical biology, we and others recently developed approaches leading to the rapid detection, identification and characterization of acylated proteins using azido- and alkynyl fatty acid analogs.

Myristoylation is the co- or post-translational attachment of myristate to N-terminal glycine residues of proteins. Very little is known about post-translational myristoylation and our new characterization efforts resulted in the demonstration that 15 or more post-translationally myristoylated proteins exist in various cell lines undergoing apoptosis and in the identification of 5 of these with life and death implications for the cell.

Palmitoylation, the modification of proteins by palmitate, is known as a key membrane tethering and cellular localization mechanism. In addition, we recently identified 21 palmitoylated proteins in mitochondria, where palmitoylation had various and profound effects on the catalytic activity and function of the modified proteins, with new implications in the regulation of metabolism.

In summary, we will present several examples illustrating how chemical biology approaches using click chemistry can catalyze the discovery of new roles for fatty acids in the regulation of cell death mechanisms and metabolism.

The association between composition of whole blood fatty acids and lifestyle factors in pregnant women

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Background: Multiple lifestyle factors are associated with maternal perinatal n-3 fatty acid levels in various blood pools, but data for whole blood are limited.

Objective: To investigate the association between levels of polyunsaturated fatty acids (PUFA) in maternal whole blood at week 24 of gestation and multiple lifestyle factors.

Methods: A total of 585 women from the novel, unselected Copenhagen Prospective Study on Asthma in Childhood (COPSAC2010) pregnancy cohort were included in this analysis. Data on socioeconomic and lifestyle factors was obtained prospectively during visits to the research unit, and fatty acid compositions of whole blood were determined. The associations between preselected PUFA variables (total n-3 PUFA, DHA, DHA+EPA, n-6/n-3 PUFA, and n-6 PUFA) and various lifestyle and socioeconomic factors were examined by univariate analysis and multivariate regression. Covariates that were significantly associated with the dependent variable in the univariate analyses were included in a stepwise backwards elimination multivariate model with a cut-off P-value of 0.15.

Results: We found independent positive significant associations between all n-3 PUFA variables and high education, high income, non-smoking, and being primiparious. The n-6 PUFA variables were not associated with any of the factors. None of the preselected PUFA variables were associated with maternal age and asthma, urban living, or gender of the child.

Conclusion: Whole blood n-3 PUFA status appears to be similar to other blood markers of n-3 PUFA with regard to associations with maternal lifestyle. Thus, when analyzing associations between n-3 PUFA-status and health, the socioeconomic and lifestyle factors that have an independent association with both blood fatty acid composition and health must be taken into consideration.

Increased omega-3 index, but no group difference after 6 weeks intake of 0.8 g EPA+DHA daily from whale oil or fish oil capsules in healthy volunteers

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Traditionally, fish oils have been studied as marine omega-3 fatty acid sources. Whale blubber was part of the traditional Eskimo diet. Whale blubber oil contains about half of the EPA and DHA content

compared to fish oils, but may still have cardioprotective effects as EFSA suggests 0.25 g EPA+DHA/day for cardiovascular disease (CVD) protection in healthy volunteers. The primary aim of the present study was to investigate if EPA+DHA uptake from whale oil (WO) and cod liver oil capsules (FO) were different in healthy subjects in terms of n-3 index (EPA+DHA percentage in erythrocytes). Inclusion criteria: healthy adults (18 yrs+). Exclusion criteria: pregnant/lactating women, intolerance for study products, known disease or medication of impact on n-3 index, bleeders/use of blood thinners, alcohol or drug abuse, heavy smoking, morbid obesity or anorexia. After news ads and telephone screening, participants were assessed for inclusion by a doctor. Fourty three subjects were randomized to whale oil (n=22) or fish oil (n=21) capsules (14 or 7 per day respectively) for 6 weeks. Marine omega-3 fatty acid intake was restricted in a two week run-in period and during the 6 week intervention period. The n-3 index increased significantly from mean±SD of 7.0±1.9 to 8.0±1.1 in WO group and 6.9±1.7 to 8.3±1.5 in FO groups respectively, which suggests a lowered risk of CVD. Urinary F2 isoprostanes were generally low though significantly increased in WO group after 6 weeks, but not compared with FO group. 25 Hydroxy vitamin D levels in serum taken late winter suggested that FO but not WO is a good source of vitamin D. In conclusion; whale oil may possibly be as good an omega-3 source as fish oil, when given in equivalent medium dosage in the present short-term bioavailability study in healthy volunteers with a relatively high baseline omega-3 intake.

Fatty acid and steroid metabolism revealed with natural and enriched stable isotopes

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High precision isotope ratio measurements reveal details of the analyte's source and metabolism that are not available from structure or concentration. Information on source is uniquely retained by intrinsic stable isotope ratios, while kinetics and pool characteristics can be obtained starting with a specific enriched compound and following the transfer of label to products. Carbon isotopes are fixed from atmospheric CO_2 via either the C3 (Calvin cycle) or C4 (Hatch-Slack) photosynthetic pathways, resulting in 13C/12C differing by about 0.003%. Most food plants operate with the C3 pathway, with corn using the C4 pathway. The acetate pool in animal cells has input from all sources of dietary carbon and thus reflects the mix of C3 and C4 plants in the diet. Three examples will be presented. (a) Retinal HUFA of corn-fed beef cattle are depleted in omega-3 HUFA and unusually enriched in omega-6 HUFA. Carbon isotope analysis reveals that the major retinal saturated and monounsaturated fatty acids originate with corn, while DHA and 22:5n-3 both originate with C3 plants that were likely to be incidental feed components. (b) Cholesterol is synthesized de novo from acetate. Steroid hormones and their metabolites reflect the dietary mix of C3 and C4 plants, with input from preformed dietary cholesterol. Thus, steroids, all of which are endogenously derived from cholesterol, should all have similar 13C/12C. Comparison of the 13C/12C of anabolic steroid metabolites to that of an endogenous reference steroid is the basis of sports antidoping steroid tests used in the Olympics and most international sporting events. (c) When used with highly enriched tracers, high precision isotope measurements are extremely sensitive and replace radioactive tracers. These techniques were instrumental in establishing the biosynthetic efficiency for the conversion of precursor PUFA linoleic and linolenic acids to highly unsaturated fatty acids in pregnant and neonate non-human primates and humans.

Therapeutic possibilities with chia and flax in obesity

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Chia and flax seeds contain the essential fatty acid, α-linolenic acid (ALA). We determined whether chia or flax attenuate the metabolic, cardiovascular and hepatic signs of a high carbohydrate, high fat (H) diet (carbohydrates 52% (w/w), fat 24% (w/w) with 25% (w/v) fructose in drinking water) in young male Wistar rats. Diets of the treatment groups were supplemented with chia or flax after 8 wk on H diet for a further 8 wk. Compared to the H rats, ALA-supplemented rats had improved insulin sensitivity and glucose tolerance, reduced visceral adiposity, decreased hepatic steatosis, reduced cardiac and hepatic inflammation and fibrosis without changes in plasma lipids or blood pressure. ALA induced lipid redistribution with lipid trafficking away from the visceral fat and liver with increased accumulation in the

heart. Stearoyl-CoA desaturase-1 inhibition was shown as an increase in the substrate concentrations together with depletion of products in the heart, liver and the adipose tissue of ALA-supplemented rats. The C18:1trans-7 was preferentially stored in the adipose tissue; the relatively inert C18:1n-9 was stored in sensitive organs such as liver and heart and C18:2n-6, the parent fatty acid of the n-6 pathway, was preferentially metabolised. Thus, ALA induces lipid redistribution associated with cardioprotection and hepatoprotection following reduced inflammatory cell infiltration. These results strongly suggest that ALA produces pharmacological responses independent of conversion to longer chain fatty acids in inflammatory conditions such as metabolic syndrome and arthritis.

Breastmilk fatty acid profile in relation to infant fat mass during the 1st year of life – Results from the INFAT-study

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Background: There is some evidence to suggest that the n-6/n-3 fatty acid ratio in early nutrition, and thus in breastmilk, could influence infant body composition.

Objective: To investigate the effect of n-3 LCPUFA supplementation and a concomitant moderate reduction of arachidonic acid (AA) intake in the diet of pregnant women/breastfeeding mothers on maternal breastmilk fatty acid composition and its relationship to infant fat mass up to 1 year of age.

Design and methods: In an open-label randomized, controlled trial, 208 healthy pregnant women either received a dietary intervention [supplementation with 1200 mg n-3 LCPUFAs per day and a dietary counseling to reduce AA intake] from 15th week of gestation until 4 months of lactation or followed their habitual diet. Breastmilk fatty acid profile was analysed at 6 weeks and 4 months postpartum. Multiple regression models adjusting for relevant confounders were performed to determine the relationship between breastmilk fatty acid composition and infant fat mass assessed by skinfold thickness measurements and abdominal sonography up to 1 year pp.

Results: Dietary intervention significantly increased breastmilk EPA and DHA content resulting in a reduced n-6/n-3 LCPUFA ratio. AA content was comparable between both groups.

DHA, EPA and n-3 LCPUFA at 6 weeks were positively related to the sum of 4 skinfolds, body fat (%) and fat mass (g) up to 1 year pp, whereas AA and n-6 LCPUFA at 6 weeks were significantly negatively associated with weight, BMI, Ponderal Index and lean body mass (g) at 6 weeks as well as with BMI at 4 months, but not at 1 year pp.

Conclusion: Breastmilk n-3 fatty acids appear to stimulate fat mass growth over the 1st year of life whereas AA seems to be involved in the regulation of overall growth especially in the early postpartum period.

The Effects of Docosahexaenoic Acid Derived 17R- and 17S-Resolvins D1 on Platelet Function in ex-vivo Diabetic and non-Diabetic Platelets

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Platelets play a role in coronary plaque rupture and ensuing thrombosis. Individuals with type 2 diabetes are at high risk for cardiovascular events.   Eicosapentaenoic acid (EPA)-derived resolvin lipid mediators have been shown to decrease platelet activation in ex-vivo human samples. Platelet spreading, platelet granulate content release, and platelet receptor conformational changes are all correlated with increased cardiovascular disease. We screened 6 adults with and 8 without type 2 diabetes to investigate the ex-vivo effects of docosahexaenoic acid (DHA)-derived 17R- and 17S-resolvin D1 alone and in combination

after adenosine diphosphophate (ADP) platelet activation and measured validated cardiovascular disease markers: granule-released p-selectin, CD40 ligand cell surface marker expression, glycoprotein IIb/IIIa platelet receptor changes, and morphologic platelet spreading. In individuals with and without diabetes, a concentration dependent reduced expression of each platelet surface activation marker occurred when 17R- and 17S-resolvin D1 were used in combination (p-value <0.05 for all fluorophores). Similarly, in both groups p-selectin expression decreased in a concentration dependent manner after treatment with either 17R- or 17S-resolvin D1 (p-value <0.05 for both). There was no differential p-selectin, CD40 ligand diminution or glycoprotein IIb/IIIa receptor changes in patients with type 2 diabetes. When compared to untreated controls, there was a significant decrease in the percentage of spread platelets after treatment with increasing concentrations of 17R-resolvin D1, untreated 67.58% vs 55.41% and 49.25% spread with 100 nm and 1000 nm 17R-resolvin D1 treatment, respectively (p-value <0.05). A significant decrease in spreading was also observed when both 17 R and 17S-resolvin D1 were used in combination, untreated 67.58% spread vs treated 47.71% spread (p-value <0.01). Our results suggest that DHA-derived resolvin lipid mediators have modest anti-platelet effects as quantified by platelet cell surface markers of activation and morphological spreading evaluation in individuals with and without type 2 diabetes. More studies should be conducted to elucidate additional anti-platelet effects of omega-3 derived metabolites.

Potential renoprotective effects of novel eicosanoids produced in diet-induced obese rats given α-linolenic acid rich diets

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Background: Arachidonic acid (ARA) derived eicosanoids influence renal hemodynamics, inflammation, and injury. However, α-linolenic acid (ALA) derived eicosanoids have yet to be isolated in the kidney or investigated for influences on renal health.

Methods: To investigate the effects of ALA on early obesity-related glomerulopathy and the renal eicosanoid lipidome, diet-induced obese rats with similar levels of obesity were provided with seven diets containing a wide range of ALA levels and n6:n3 ratios.Diets and n6:n3 ratio: Canola-Flax-(1:1), Canola-(2:1), High-Oleic-Canola+Canola-(3:1), High-Oleic-Canola-(7:1), Soy-(7:1), Lard-(9:1), Safflower-(75:1).

Results: Of 64 eicosanoids scanned by LC-MS/MS, 33 were present at detectable levels. Prostanoids have been associated with later stages of renal disease and glomerulomegaly. In this early stage of renal pathology, no linoleic acid (LA) or ARA derived eicosanoids were associated with glomerular volume (GV). Conversely, ALA, eicosapentaenoic and docosahexaenoic acid derived eicosanoids were inversely correlated with GV: 9-hydroxyoctadecatrienoic acid-(9-HOTrE)-(r=-0.34), 13-HOTrE-(r=-0.31), 5-hydroxyeicosapentanoic acid-(5-HEPE)-(r=-0.33), and 4-hydroxydocosahexanoic acid-(4-HDOHE)-(r=-0.30).

The canola-flax group had greater 9-HOTrE, 13-HOTrE, and 5-HEPE levels than the high-oleic-canola, lard, and safflower groups, and higher levels of 4-HDOHE than the safflower group.

When dietary LA levels were similar but ALA was elevated, significantly greater 9-HOTrE, 13-HOTrE, and 5-HEPE quantities were observed. Interestingly, when ALA quantities were comparable and LA levels were 2.5 times higher (High-Oleic-Canola+Canola vs. Soy), renal HOTrE levels were 3-fold greater. When n6:n3 were identical, but LA and ALA were 2.7 times higher (Soy vs. High-Oleic-Canola), 9-HOTrE and 13-HOTrE quantities were 5 and 12 times higher, respectively.

Using logistic regression, renal HOTrE levels could be predicted by renal ALA (HOTrE=-6.7+28.03(ALA)) and LA (HOTrE=-7.88+0.638(LA)).

Conclusions: Novel renal eicosanoids produced in vivo with ALA enriched diets were identified. HOTrEs, 5-HEPE and 4-HDOHE were associated with protection from glomerulomegaly and may be earlier markers of glomerular health status than prostanoids. HOTrEs not only are influenced by dietary ALA, but LA as well.

Prenatal docosahexaenoic acid (DHA) and pregnancy outcomes

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Background: Observational and some experimental studies suggest that n-3 fatty acids may increase gestation and birth weight, but overall results are mixed.

Objective: We hypothesized that women assigned to DHA would have higher red blood cell phospholipid (RBC-PL) DHA, longer gestation and larger infants.

Methods: Women were enrolled (n=350) in a Phase III randomized placebo-controlled clinical trial to evaluate the safety and efficacy of DHA supplementation (mean 14 wks gestation until delivery) on pregnancy outcome (reported here) and infant/toddler development (NCT00266825). Algal oil DHA (600 mg) or placebo (soybean and corn oil) were provided in 3 orange-flavored capsules/day (Martek Biosciences, Inc.). DHA (wt% total fatty acids) in maternal and cord RBC-PL was determined at enrollment (maternal) and delivery (maternal and infant). Delivery gestational age was based on ultrasound in the second trimester of pregnancy. Weight, length and head circumference were obtained at birth. All subjects for whom data were available at delivery (n=299) were included in an intent-to-treat analysis and mean differences compared by one-sided p-values.

Results: Women in both groups consumed similar numbers of capsules (mean 2.6/d) and had similar numbers of maternal and infant serious adverse events. Maternal and cord RBC-PL at delivery were increased in the DHA compared with the placebo group (7.3 vs. 4.7%, $p<0.001$ and 7.3 vs. 5.9%, $p<0.001$, respectively). DHA increased mean gestational age from 272.7 to 275.6 days ($p<0.04$), and birth weight, length and head circumference from 3187 to 3359 g, ($p <0.005$), 49.0 to 49.7 cm ($p<0.02$); and 33.7 to 34.2 cm ($p<0.01$), respectively.

Conclusion: DHA supplementation at this level is safe, Prenatal supplementation increased gestation duration, DHA status markers in mother and newborn, birth weight, length, and head circumference. (Supported by 1R01 HD047315).

Clinical impact of ruminant trans fatty acids

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Since the 90's, a huge literature is available on the effects of industrially-produced trans fatty acids (IP-TFA) on cardiovascular risk factors. The effects of ruminant trans fatty acids (R-TFA) have only been studied recently. This might be due to (i) the low level of R-TFA intake compared to IP-TFA in several countries like North America and the northern part of Europe or (ii) the lack of possibility to obtain R-TFA rich milk fat.

The Nurse Health study already suggested that R-TFA intake was not associated with Coronary heart disease. Similarly, other epidemiological studies also indicated the lack of association between R-TFA intake and cardiovascular risk.

Intervention studies carried out in Canada and France were published at the beginning of the present Century. These data suggested that, at least at usual levels of consumption, there is no association between R-TFA intake and cholesterol-dependant cardiovascular risk factors, but men and women appeared to present different patterns.

To draw definitive conclusion, we conducted a meta-analysis including all the intervention studies in healthy volunteers with at least one experimental group with documented R-TFA intake. From thirteen studies that met all our selection criteria, we extracted data from one or several groups, yielding to twenty-two observations. Daily intakes of R-TFA ranged from 0.12 to 4.19 % of total energy intake.

For each trial, were extracted or calculated when necessary, the change between baseline and the end of the intervention period in both ratios of Total Cholesterol to HDL-C (ΔTC/HDL) and of LDL-C to HDL-C (ΔLDL/HDL), diet characteristics (including the intake of total R- trans C18:1), participant characteristics (age, BMI and lipoprotein cholesterol concentrations at baseline). Linear regression analyses were

performed and results indicate that the qualities of regressions between R-TFA dose intake and both ΔTC/HDL and ΔLDL/HDL were very poor.

This study suggests that R-TFA intake does not significantly impact the ratio of TC/HDL-C, a robust marker to estimate cardiovascular risk. However, further analysis such as multivariate analysis including confounders would be relevant to confirm these results.

Mitochondrial β-oxidation is necessary for the disappearance of 14C-EPA upon entry into the brain: an in vivo free-living intravenous infusion study

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Background: Eicosapentaenoic acid (EPA) is currently under investigation as a potential treatment for neurological disorders such as bipolar disorder and major depression; even though, in the brain, its function is poorly understood and the levels of EPA are 250-fold lower than docosahexaenoic acid (DHA). Previously, we demonstrated that EPA was more readily β-oxidized compared to DHA and palmitate upon entry from the plasma in situ and upon intracerebroventricular injection in vivo. Objective: To examine if mitochondrial β-oxidation is necessary for maintaining low EPA levels in brain phospholipids. Procedures: 150 µCi/kg of 14C-radiolabeled palmitate, DHA or EPA was administered intravenously to rats after pre-treatment with vehicle or methyl palmoxirate (MEP), a carnitine palmitoyltransferase-1 antagonist. Free-living rats were infused at steady state for 5 minutes via the tail vein and blood samples were collected throughout infusion via jugular vein catheter. After 5 minutes, rats were subjected to high-energy, head-focused microwave irradiation and brains were collected. Aqueous (marker of β-oxidation) and total lipid fractions were counted. Preliminary results: Radioactivity in the aqueous fraction decreased significantly in MEP-treated brain compared to vehicle controls (palmitate: 3.8-fold; DHA: 1.5-fold; EPA: 2.2-fold). This was accompanied by a significant increase in total lipid radioactivity of MEP-treated brains as compared to controls (palmitate: 1.2-fold; DHA: 1.1-fold; EPA: 1.5-fold). Future analysis: Radioactivity in various neutral lipids and phospholipid fractions will be analyzed and the identities of radiolabeled fatty acids will be determined. Conclusion: Mitochondrial β-oxidation plays an important role in the catabolism of EPA upon entry into the brain.

Polyunsaturated Fatty Acids Modify Phospholipid Structure and AKT Signaling in Cancer Cells

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Phospholipids are crucial components of cellular membranes as well as signaling molecules, with fatty acids at the sn-1 and sn-2 position of the glycerol backbone, and choline, ethanolamine, serine or inositol at the sn-3 position. AKT is a serine-threonine protein kinase that plays important roles in cell growth, proliferation and apoptosis. It is well documented that AKT activation requires its binding to phosphatidylinositol phosphates (PIPs) with phosphate groups at positions 3, 4 [i.e. PI(3,4)P2] and 3,4,5 [i.e. PI(3,4,5)P3] on the inositol ring. However, it is unclear whether fatty acids at the sn-1 and sn-2 position can affect the ability of PIPs to activate AKT. Here we show that dietary polyunsaturated fatty acids (PUFA) modify phospholipid structure. ω3 PUFA such as docosahexaenoic acid (DHA) can replace the fatty acid at the sn-2 position of the glycerol backbone, thereby generating different species of phospholipids. DHA also inhibits AKTT308 but not AKTS473 phosphorylation, alters PI(3,4,5)P3 and phospho-AKTS473 (pAKTS473) protein localization, decreases BAD-AKT interaction, and suppresses tumor growth. Our study suggests that dietary fat, through the structural modification of phospholipids, can affect the AKT signaling pathway that is critical in the development of human cancers.

Arachidonic Acid Enhances TNF-Alpha-Induced NF-KB Signaling Studied in Human Breast Cancer Cell Line and Rat Mammary Tumors

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Our Lab previously found that arachidonic acid (AA, 20:4n-6) levels are 10 times higher in mammary tumor tissue than in the normal mammary gland, and are positively correlated with the tumor weight. However, the mechanism is not clear. We then proposed AA may enhance nuclear factor kappaB (NF-KB) activation studied in human breast cancer cell line MCF-7 and rat mammary tumors. We hypothesized that AA enhanced tumor necrosis factor-alpha (TNF-A)-induced NF-KB signaling for the growth the breast cancer cells. We also studied the correlation between NF-KB signaling and tumor weight in rat mammary tumor. MCF-7 were pretreated with 0, 10, 50 and 100 M AA for 48 hours, then were stimulated with TNF- A. Western blot analysis was performed on whole cells, cytosol and nuclear fractions for the NF-KB signaling protein expression. AA supplementation resulted in increasing pAkt /Akt levels, IK-B degradation, nuclear p65 expression in MCF-7. In study of rat mammary tumors, the tumor weights were positively correlated with pAkt/Akt ratio (r = 0.6899, p < 0.0001), were inversely correlated with IK-Ba expression (r = 0.6039, p = 0.0037), were positively correlated with nuclear p65 expression (r = 0.5868, p = 0.002) and were positive correlated with c-Myc expression (r = 0.6036, p = 0.0023). It was concluded that AA enhanced NF-KB induced p-Akt signaling resulted in increasing IK-Ba degradation and nuclear p65 expression for the growth of breast cancer.

13C-DHA Metabolism Before and After EPA + DHA Supplementation in Humans

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Introduction: EPA and DHA levels in blood usually correlate positively with fish intake but reach a plateau at high EPA + DHA intakes (> 3 g/d). The question of whether DHA is more beta-oxidized at a high EPA and DHA intake was investigated in this study. Objective: Evaluate carbon-13 DHA (13C-DHA) metabolism before and during the last month of a 5 month supplementation with EPA + DHA in humans.

Methods: Forty healthy participants were recruited. Pre-supplementation (control): A single oral dose of 50 mg of 13C-DHA was given to the participants at breakfast, and its appearance in plasma and beta-oxidation was monitored for 28 days. A supplement (3.2 g/d in EPA + DHA) was given to the participants daily for 5 months. Post-supplement: In the last 28 days of the supplementation, a single oral dose of 50 mg of 13C-DHA was given to the participants to follow its metabolism.

Results: Plasma DHA and EPA were 2.5 times higher post-supplement than control (p < 0.01). 13C-DHA concentration in plasma reached its maximum 6 h post-intake in before and after the supplement. Post-supplement, plasma 13C-DHA concentrations were 26 to 72 % lower at 6 h, 24 h, 7 d, 14 d and 21 d (p < 0.05) compared to pre-supplementation. Post-supplement, cumulative beta-oxidation was 1.3 to 1.9 times higher at 6 h, 8 h, 24 h, 7 d, 14 d and 21 d (p < 0.05) compared to pre-supplementation.

Conclusion: High dose DHA supplementation increases 13C-DHA beta-oxidation and lowers its appearance in plasma, which helps explain the blood DHA plateau attained with high DHA intake.

LCPUFA Supplementation in Infancy Affects Measures of Childhood Cognition

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Background: LCPUFA status and supplementation in infancy has been reported to affect cognitive and developmental outcomes positively. Docosahexaenoic acid (DHA), which is found in high concentration in the brain, is specifically thought to affect cognitive function.

Objective: To determine whether LCPUFA supplementation (and DHA dose) affects children's cognitive performance from 18 mo to 5 yr of age.

Design/Methods: A double-blind, randomized, controlled, parallel-group prospective trial was conducted. At delivery infants were randomly assigned to formulas varying in DHA content: 0.00% (control), 0.32%, 0.64% and 0.96% DHA. Arachidonic acid was present at 0.64% in the supplemented formulas but not in the control. Formulas were fed to 12 mo of age. Cognitive outcomes included the Bayley Scales of Infant Development II (BSID: 18 mo), the Dimensional Change Card Sort (DCCS: 36, 42, 48, 60 mo), Bear/Dragon Go/No-Go, Red/Yellow Stroop, and Day/Night Stroop tasks (36, 42, 48 mo), and the Peabody Picture Vocabulary Test (PPVT: 60 mo).

Results: Seventy of 86 infants followed over the long-term remained in the study through 60 mos. Analyses included Formula and (for repeated tests) Age. No significant differences emerged on the BSID, Bear-Dragon or Red-Yellow Stroop tasks. Analysis of the DCCS yielded effects for Formula (p=.017) and Age X Formula (p=.009); 0.32% and 0.64% groups showed enhanced developmental gains from 3 to 5 yr across Pre-and Post-Switch phases of the task. This interaction persisted (p=.006) when supplemented groups were combined and tested against controls. Analyses of total correct on the Day-Night Stroop yielded a Formula effect (p=.035); the 0.64% and 0.96% groups showed better performance across all ages and controls and the 0.32% group. Analysis of the PPVT yielded a Formula effect(p=.017), with supplemented groups scoring above controls; this effect persisted (p=.009) when formula groups were combined and tested against controls.

Docosahexaenoic acid, brain aging and Alzheimer's disease: Reconciling the evidence

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The conceptual links between the relatively high prevalence of Alzheimer's disease (AD) in Western countries, the role of DHA in brain development, and the longstanding concerns about inadequate intake of omega-3 fatty acids in these same Western countries have together focussed considerable interest on whether raising DHA intake could decrease the risk of AD and/or the progression from mild cognitive impairment towards AD dementia. On the positive side, prospective epidemiological studies support a robust link between habitually high intake of fish and/or DHA and lower risk of AD. Further support comes from significant progress in understanding the neurophysiology of DHA at the cellular and lipidomic levels, and in animal models of neurodegenerative disease. Hence, there is considerable momentum reinforcing the concept of a protective effect of DHA on cognition in the elderly. Unfortunately, the outcome of AD trials with DHA supplements alone, or the more common mix of DHA+EPA in fish oil capsules, has consistently failed to produce the anticipated beneficial results. In earlier AD trials, insufficient duration may well have been a factor in the lack of cognitive benefit of DHA alone or DHA+EPA, but this was probably not the case in a recent large trial with negative outcome. Hence, there is a problem in the human studies on AD, i.e. the negative results of clinical trials with DHA supplements don't agree with the largely protective link of fish and DHA observed in prospective epidemiological studies. The focus of this talk will therefore be on discussing two themes that may bridge this disconnect: (i) human studies of DHA levels in AD brain and plasma, and (ii) changing DHA homeostasis in the elderly. The emerging recognition that two important risk factors for AD, i.e. aging itself and apoE4, both change DHA metabolism may shed light on this disconnect in a way that presently has no obvious counterpart in animal or in vitro studies.

Oxidative Stress in Homozygous Sickle Cell Patients is Not Aggravated by Supplementation With Docoshexaenoic and Eicosapentaenoic Acids

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Background: Blood cells of patients with sickle cell disease (SCD) have reduced levels of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. This abnormality is thought to be a factor for the increased tendency of blood cells of SCD patients to aggregate and adhere to vascular endothelium and subsequently precipitate vas-occlusive crisis and organ damage. Hence, supplementation with DHA

and EPA may provide clinical benefits. However, sickle cell patients are under oxidative stress and this could be aggravated by supplementation.

Objectives: To investigate the effect of DHA and EPA on anti-oxidant status of sickle cell patients. .

Procedure: Sudanese homozygous sickle cell patients (n=23), aged 2 to 18 years, were given one (2-4 year old), two (5-10), three (11-16) and four (≥ 17) omega 3 fatty acid capsule containing 277.8 mg DHA and 39.0 mg EPA for one year. Plasma alpha-tocopherol (vit E), and red blood cell antioxidant enzymes and dimethy acetals (marker of plasmalogen status) were analysed at baseline and six-month supplementation. Vit E was assessed in a sub-group of patients after one year.

Results: Supplementation increased DHA and EPA three- fold in red cell choline (CPG) and ethanolamine (EPG) phosphoglycerides ($p<0.001$). The activities of Se-GPx (31.3 ± 17.7 vs 42.2 ± 13.5 IU/gHb, $p<0.001$) and Cu/Zn –SOD (9.9 ± 4.6 vs 12.4 ± 4.6 IU/gHb, $p<0.01$) were lower after six months of intervention compared with baseline. There was no difference in percent total dimethylacetals in CPG (0.6 ± 0.1 vs 0.6 ± 0.1, $p>0.05$) and EPG (13.6 ± 1.1 vs 13.9 ± 1.6, $p>0.05$) or concentrations of plasma tocopherol (10.6 ± 2.1 vs 10.2 ± 3.5 µmol/l, $p>0.05$) between the two time points. Plasma vit E concentration increased after one year supplementation (14.4 ± 3.2 vs 10.2 ± 2.6 µmol/l, $p<0.001$, n=17).

Conclusion: Oxidative stress was not aggravated by the given dose of DHA and EPA. Hence, it should be safe to supplement sickle cell patients to help ameliorate membrane abnormality and vaso-occlusive crisis.

Symposia on fatty acids and early childhood, Omega-3 fatty acid supplementation in obese adolescents - on vascular function, inflammation, insulin sensitivity and tissue fatty acid composition udy

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Compared with normal-weight adolescents, obese subjects show lower concentrations of omega-3 polyunsaturated long chain fatty acids (LCPUFAs) in serum phospholipids. We wanted to assess whether supplementation of omega-3 LCPUFAs increases the omega-3 LCPUFA concentration in serum, skeletal muscle and adipose tissue, improves vascular function and morphology and lowers inflammation in obese adolescents. We also wanted to investigate effects on glucose and insulin homeostasis. Methods: 25 obese adolescents (14 females, 11 males, age 15.7 ± 1.0 yrs, BMI 33.8 ± 3.9) were randomized to intake of capsules containing either 1.2 g/day of omega-3 LCPUFAs or placebo for 3 months. The study was performed in a double blind, crossover design with 6 weeks washout period. Anthropometry, blood pressure measurements and fasting blood samples were obtained before and after each treatment period. Vascular structure and function were measured, intravenous glucose tolerance test (IVGTT) and euglycemic-hyperinsulinemic clamp were performed, and adipose tissue and skeletal muscle biopsies were obtained after each treatment period.

The concentrations of EPA, DHA and total omega-3 PUFA in serum, muscle and adipose tissue increased in both sexes with omega-3 LCPUFA supplementation. Reactive hyperemia response was improved ($p<0.01$) and lymphocytes, monocytes, TNF-α, IL-6 and IL-1β lowered after omega-3 LCPUFA supplementation. No difference in total cholesterol, triacylglycerol, HDL cholesterol, anthropometry, blood pressure, pulse wave velocity or vascular structure could be found. In the females, omega-3 LCPUFA supplementation improved glucose tolerance by 39% ($p=0.04$) and restored insulin concentration by 34% ($p=0.02$) during IVGTT. Insulin sensitivity improved 17% ($p=0.07$). In males, none of these parameters was influenced by omega-3 supplementation.

Daily supplementation with omega-3 LCPUFA capsules to obese adolescents increases serum, skeletal muscle and adipose tissue omega-3 LCPUFA concentration, improves vascular function and lowers the degree of inflammation. It also improves glucose and insulin homeostasis in obese girls without influencing body weight.

Gut Bacteria Engineered to Express N-acyl-phosphatidylethanolamine Reduce Weight Gain in High-Fat Fed Mice

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Differences in gut commensal microbiota appear to contribute to an individual's propensity to be obese, so that manipulating this microbiota may help treat obesity. Since the identities of leaness-promoting bacteria are unknown, we tested an alternative strategy: genetically engineering gut bacteria to secrete mediators known to reduce fat intake. N-acyl-phosphatidylethanolamines (NAPEs) are normally synthesized in the small intestine and their metabolites, N-acyl ethanolamides (NAEs), increase satiety and decrease food intake. We transformed the probiotic E.coli, Nissle 1917 (EcN), with expression plasmids encoding At1g78690p , a recently cloned acyltransferase from Arabidopsis thaliana that catalyzes NAPE formation. EcN transformed with At1g78690 (pAT-EcN) synthesized ~100-fold more NAPEs than EcN transformed with empty plasmid vector (pEmpty-EcN). Four groups of C57BL6 mice (10 mice each) received one of four treatments in their drinking water: no additive (untreated), 0.125% gelatin (vehicle), $5x10^9$ cfu pAT-EcN/ml or $5x10^9$ cfu pEmpty-EcN/ml. All groups were also fed a high-fat diet ad libitum for 8 weeks. Koalin consumption, a measure of gastric distress in mice, was similar in all four groups. Weight gain, %body fat, and calories consumed were significantly lower in the pAT-EcN treated mice compared to other groups from the third treatment week on. After 8 weeks treatment, pAT-EcN treated animals had gained 29% less weight (7.84 g) than untreated animals (11.0 g, $p<0.05$), while weight gain by vehicle and pEmpty-EcN treated mice were similar to untreated mice. %Body fat for pAT-EcN mice was only 21%, compared to 27% in the untreated mice. We then stopped treatment of drinking water while continuing high-fat feeding for 4 additional weeks. Post-treatment body weight gain for mice previously administered pAT-EcN was 1.7 g compared to 2.9 g for untreated mice. These findings suggest that consumption of probiotics engineered to express NAPE may be a useful treatment strategy for obesity.

Dietary n-3 polyunsaturated fatty acid regulation of adipokines and inflammatory mediators in adipocyte-macrophage paracrine interactions in vitro

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In obesity, paracrine interactions between adipocytes and infiltrating macrophages in adipose tissue generate inflammation and related complications. Interestingly, the long chain n-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) are known to exert anti-inflammatory effects and thus may represent a strategy to reduce synthesis and secretion of pro-inflammatory adipokines, such as tumour necrosis factor (TNFα), monocyte chemoattractant protein 1 (MCP-1) and interleukin 6 (IL-6) from obese adipose tissue. To address this, we developed an in vitro murine co-culture model that mimics the adipose tissue macrophage infiltration found in the db/db mouse model of obesity. Mature murine 3T3-L1 adipocytes were incubated with RAW 264.7 macrophages in direct contact, or separated by a trans-well membrane, in the presence of 125 µM EPA, DHA, or palmitic acid (PA), all complexed to albumin, or albumin alone (control). After 12 h, IL-6 and MCP-1 secreted protein was markedly suppressed in DHA (74%, 58% respectively) and EPA (33%, 49% respectively) treated contact co-cultures, compared to PA and control ($p<0.05$). Similar results were found in the trans-well system, although in all fatty acid groups, adipokine secretion was nearly two-fold lower from the trans-well co-culture, emphasizing the importance of direct adipocyte-macrophage contact in paracrine interactions. The trans-well co-culture system allowed for isolation of adipocytes to measure mRNA expression of pro-inflammatory mediators. While PA increased ($p<0.05$) IL-6 mRNA expression in adipocytes relative to control, DHA decreased ($p<0.05$) the mRNA expression of TNFα, IL-6, toll-like receptor 4 (TLR-4) and nuclear factor-κB (NFκB) compared to adipocytes treated with PA or control. Overall, these data demonstrate that long-chain n-3 PUFA can decrease pro-inflammatory adipokine secretion and mRNA expression of various pro-inflammatory mediators and thus may provide a beneficial strategy to reduce inflammation in an obese state characterized by macrophage infiltration into adipose tissue. (Funded by NSERC).

Eating the right amount of fish: Inverted U-shape association between fish consumption and cognitive performance and academic achievement in Dutch adolescents

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Fish consumption has shown its benefits for cognitive functioning in the elderly or children with disorders (e.g,, autism, ADHD), but has rarely been investigated in relation to cognitive performance and school performance of healthy adolescents. Therefore an observational study in 700 Dutch high school students aged 12-18 years was executed. Fish consumption data, end term grades, scores on the Amsterdam Vocabulary Test, and scores on the Youth Self-Report were collected. Results revealed that 13.6% of the Dutch adolescents never ate fish, 6.4% met national guidelines, 16.9% reached half of the recommended amount, and 63.1% did eat fish but too little to meet at least half of the recommended amount. Analysis of variance, controlled for relevant covariates, showed significant differences between the four fish consumption groups in vocabulary ($p= 0.05$). A trend for significance was found for end term grades ($p= 0.07$). Contrast analyses demonstrated significant quadratic associations between fish consumption and vocabulary ($p= 0.01$) and end term grades ($p= 0.01$). Thus, our findings suggest that irrespective of sex, age, and educational track, the association between fish consumption and cognitive performance and academic achievement in adolescents consists of an inverted U-shape. Higher fish intake was associated with more advanced vocabulary and higher end term grades. However, eating more fish than the described recommended amount seemed no longer beneficial. The differences found between the groups (e.g. for academic performance) could be relevant for educational practice. The difference in z-score between the 1575-3150 mg fish group and the highest fish consumption group equals 0.23 points differences on a 10 point scale (Dutch grades are not given in letters, but in numbers between 0-10). This difference in fish consumption could therefore account for the difference between passing or failing.

Protective effect of dairy fat on brain DHA levels of young rats born from ALA-deficient or ALA-rich mothers

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DHA is the major brain FA and omega-3 deficiencies during gestation/lactation could have dramatic impacts on health during adulthood.

Objectives: To evaluate: 1/ the impact of dietary deficient or ALA-rich diets during gestation/lactation on the brain DHA levels of post-weaning young males submitted to deficient or ALA rich-diets; 2/ the specific impact of different matrix: butter fat compared to rapeseed oil diets to restore or maintain brain DHA levels in young rats born from deficient or ALA-rich dams.

Procedure: Two groups of dams were fed during gestation and lactation with either a deficient ALA-palm diet containing minimal ALA level (0.4%) or a protective ALA-rich (8%) pure Rapeseed oil diet.

After weaning, 3groups of young males born from deficient and ALA-rich dams received a 10%fat diet for 6 weeks, either (i) as ALA-deficient palm diet (ALA0.4%), (ii) ALA-low butter diet (ALA0.8%), (iii) ALA-rich rapeseed diet (ALA8%).

Results/Conclusions: - New-born and weaning pups from deficient dams showed brain DHA levels 2 times lower than those from ALA-rich-dams. - The brain DHA levels of the post weaning rats were more dependent of the dams status than of their own diet: an ALA-rich diet during gestation and lactation is protecting against ALA deficiency during post-weaning growth, while an ALA-rich diet post-weaning allow a recovery for those born from deficient-dams, but never reach the values of those born from ALA-rich-dams.

- Butter fat, despite 10times less ALA than rapeseed oil (0.8%vs8%), is as efficient as rapeseed to restore the Brain DHA level in young rats born from deficient-dams and to maintain similar levels for those born from ALA-rich-dams. The same low n6/n3ratio in these two type of fats (3 while 25 for palm) and the complexity of the composition of dairy fat could be part of its protective effect. Partially granted by Lactalis

Alpha Linolenic Acid Regulates Survival in TNF-Induced Apoptosis in Cardiomyocytes

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Cellular mechanisms presiding over cardiac beneficial effects induced by n-3 polyunsaturated fatty acids remain to be fully understood, in spite of several studies carried out on marine-derived docosahexaenoic (DHA) and eicosapentaenoic acid (EPA) and plant-derived alpha-linolenic acid (ALA). The present study demonstrates that ALA is a very potent inhibitor of the apoptosis induced by the tumor necrosis factor (TNF) in cardiomyocytes. Indeed, after short exposure to TNF, survival cascades are promoted in cardiac cells, while a long-lasting cytokine stimulus causes apoptosis. ALA pre-treatment inhibits the onset of the apoptosis in cardiomyocytes through a mechanism involving caveolae. In fact, in the presence of ALA, caveolin-3 expression is enhanced and the internalization of the TNF receptor, located into the caveolae, is inhibited determining the abortion of the apoptotic vs. survival cascade. This study unveils a novel mechanism involving the TNF receptor and explains the n-3 PUFA cardioprotective effects. The anti-TNF ALA effects have been also confirmed in an in vivo model of hereditary cardiomyopathy suggesting that a "membrane lipid therapy" can be set up to prevent cardiac degenerative diseases

Fish oil improves mitochondrial function in brains of aged mice

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The brain consumes disproportionally high amounts of oxygen due to its high metabolic activity. In case of a deficit of antioxidants and antioxidant cellular mechanisms the high turnover of oxygen results in the production of reactive oxygen species and ensuing damage of essential biomolecules. Those deleterious effects accumulate especially in differentiated tissues like the brain. As a major consequence, perturbations of the energy metabolism including mitochondrial dysfunction culminate in functional deficits. With the increasing average life span of humans, age-related cognitive disorders such as Alzheimer disease (AD) are a major health concern in our societies. Strategies for long-term prevention from mitochondrial dysfunction including sufficient supply of essential nutrients may also delay the onset of age-related neurodegenerative diseases. The present study investigated the effects of orally administered DHA- and EPA-rich fish oil (FO) on mitochondrial function in brains of young (3 months) and aged (24 months) NMRI-mice. Neuroprotective properties of FO were assessed ex vivo after 21 days in dissociated brain cells (DBC) and isolated mitochondria (mito). DHA levels were significantly lower in brains of aged mice and this deficiency was compensated by FO administration. Isolated DBC and mito from aged mice showed significant lower ATP levels and reduced respiration, respectively. FO restored the age-related decrease in respiration, especially complex I+II and IV activities of the mitochondrial respiration chain were improved. Moreover, FO promoted the production of ATP. Recent data identified DHA-mediated increases in sAPPalpha, which were associated with protection of mitochondrial function in vitro (BBA 1808(2011)236). Accordingly, we currently investigate if sAPPalpha and/or DHA-derived metabolites such as NPD-1 are responsible for the observed beneficial effects of FO against age-related mitochondrial dysfunction in vivo. Our findings provide new mechanisms underlying the neuroprotective actions of polyunsaturated fatty acids and identified FO as promising neutraceutical to delay age-related cerebral alterations.

Effect of EPA+DHA supplementation on blood lipid profile of British children of a poor socio-economic background

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Many children have unfavourable blood lipid profiles and high blood pressure, which triggers development of atherosclerosis early in life and predispose them for high risk of CVD in adulthood. It is established that higher doses of very-long-chain omega-3 fatty acids EPA+DHA from fish reduce blood triglyceride levels, but effects of EPA+DHA supplementation on blood lipid profiles in apparently healthy children have not been investigated before. The current study aims to investigate the effect of EPA+DHA on blood lipid profile of British children from a poor socio-economic background, which is a secondary outcome of the study. Forty children aged 9-12 y were matched in pairs for age, sex and maternal education and randomly assigned to intervention or control treatment. One group received daily a margarine enriched with either 1.2 g/d of EPA+DHA (intervention) or oleic acid (control). The study had a parallel design and duration of 3 months. At baseline and end of intervention, fasting blood samples were drawn and analyzed on fatty acid composition of plasma lipids and red blood cell (RBC) phospholipids and on concentration of blood lipids. Baseline blood lipid concentrations were on average 0.77± 0.37 (SD) mmol/L for triglycerides and 3.83±0.69 mmol/L for total cholesterol, and did not differ between the two treatment groups. Preliminary analyses show significant increases in proportions of EPA and DHA in plasma lipids and RBCs in the intervention group, confirming good compliance. Results on blood lipid concentration will be presented and discussed.

DHA, Lipid Rafts and Breast Cancer

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Long chain n-3 polyunsaturated fatty acids (LCPUFA) have been shown to possess anti-carcinogenic properties in mammary cancers, both in vitro and in vivo. Docosahexanoic acid (DHA) and eicosapentaenoic (EPA) were found to significantly inhibit the growth of human breast cancer cell lines (MDA-MB-231, MCF-7 and SKBr-3), but not a non-transformed MCF-12A breast cell line, in a dose dependent fashion. LCPUFA are rapidly incorporated into the microdomains of the cell membrane (lipid rafts) where important death and growth receptors and signals are located. Studies from our laboratory have demonstrated that the incorporation of the DHA and EPA into lipid rafts is associated with reduced epidermal growth factor receptor and increased CD95 (Fas) in plasma membrane rafts. These changes were associated with post membrane changes in the signaling pathways and cell growth and apoptosis, thereby providing possible membrane-mediated mechanisms for the effects of n-3 LCPUFA on the survival of human breast cancer cells. The cell membrane is also the target for doxorubincin (DOX or adriamycin) one of the drugs of choice for treatment of highly invasive metabolically active breast tumors. In vitro, DHA, but not EPA, potentiates the cytotoxic effects of DOX in estrogen receptor negative MDA-MB-231 breast cancer cells. Our recent work has demonstrated that surface expression and lipid raft CD95 co-localization is increased by DHA+DOX treatment compared to a control fatty acid treatment. These results suggest that the effects of DHA on the efficacy of DOX treatment on human breast cancer cells may be mediated in part by CD95-induced apoptosis in MDA-MB-231 cells.

Functional links between arachidonic acid and endocannabinoids in the regulation of inflammation

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Arachidonic acid is a fatty acid involved in most, if not all physiological processes. Its metabolism into pro- and anti-inflammatory eicosanoids (e.g. leukotrienes, prostaglandins, lipoxins) either results in enhanced or decreased inflammation. Other bioactive lipids play key roles during inflammation. Among them are the endocannabinoids, which consist of a fatty acid linked to a molecule of glycerol or a molecule of ethanolamine. The resulting glyceryl-esters (e.g. 2-arachidonoyl-glycerol) and ethanolamides (e.g.

arachidonyl-ethanolamide) have been linked to the regulation of inflammation by activating the specific G-protein-coupled receptors CB1 and CB2. Endocannabinoids are biosynthesized on demand and are hydrolyzed rapidly to fatty acids. While the pharmacological or genetic inhibition of cannabinoid receptors supports an anti-inflammatory role of endocannabinoids, the latter induce pro- and anti-inflammatory effects. We believe this is related to 1) their metabolism by eicosanoid biosynthetic enzymes; and 2) their hydrolysis into arachidonic acid and the subsequent synthesis of eicosanoids. The resulting lipidome consists of numerous bioactive lipids with either pro- or anti-inflammatory effects. Interestingly, while endocannabinoids can serve as a source of arachidonic acid, fatty acid intake modulates endocannabinoid levels in the tissues. Recent evidence supports this functional link between arachidonic acid and endocannabinoids as they play a key role in the regulation of inflammation. In this regard, it remains unclear whether we should enhance or reduce arachidonic acid levels/intake in order to limit the onset of inflammation and to promote its resolution. Key findings regarding the functional link between endocannabinoids and arachidonic acid will be presented.

Fatty acids, lipids and cardiovascular HDL

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Levels of plasma high density lipoprotein cholesterol (HDL-C) correlate generally and directly with protection against coronary heart disease, yet the level of cholesterol itself in HDL particles is frequently a poor predictor of HDL function and protection. HDL are thought to protect against atherosclerosis for numerous reasons, including removing excess cholesterol from cells and reducing artery wall inflammation. Transgenic animal studies to raise the main protein of HDL, apolipoprotein A-I, and thereby HDL-C levels show marked reduction in atherosclerosis, providing the best evidence so far of the benefit of raising HDL-C specifically. No lifestyle or therapeutic maneuvers, however, so far raise HDL-C specifically enough to allow this hypothesis to be tested in humans. Clinical trials with niacin to raise HDL-C, among other effects on lipoproteins, have had mixed results, and more definitive trials are ongoing. Agents that raise HDL-C by inhibiting cholesteryl ester transfer protein are also being investigated for potential benefit. This presentation will review the potential beneficial actions of HDL, other markers of HDL function that may be more informative than HDL-C, and maneuvers to raise HDL that are currently available or under development

Linoleic acid and inflammation: evidence based research from clinical studies

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A systematic review of randomized controlled trials that permitted the assessment of dietary linoleic acid (LA) on biological markers of chronic inflammation among the healthy non-infant population was conducted. A search of the English and non-English literature using MEDLINE, the Cochrane Controlled Trials Register and EMBASE was conducted to identify relevant articles. Fifteen studies (eight parallel and seven cross-over) met inclusion criteria. None of the studies reported significant findings for a wide variety of inflammatory markers including C-reactive protein, fibrinogen, plasminogen activator inhibitor type 1, cytokines, soluble vascular adhesion molecules or tissue necrosis factor-alpha. The only significant outcome measures reported for higher LA intakes were greater excretion of prostaglandin E2 and lower excretion of 2,3-dinor-thromboxane B2 in one study 1 and higher excretion of tetranorprostanedioic acid in another. However, both authors observed that these effects were not an indication of increased inflammation. It is concluded that virtually no evidence is available from randomized, controlled intervention studies among healthy, non-infant humans to show that variations in the level of LA in the diet affects in vivo inflammation in healthy humans.

Saturated fats: Guilty without trial?

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Recommendations to prevent cardiovascular disease (CVD) have rested heavily on a reduction in saturated fat intake. Recent evidence has called into question this mantra. Confounding influences that occur as a result of variations in the effects of specific saturated fatty acids, replacement by other macronutrients, obesity state and a myriad of lifestyle factors has called the saturated fat hypothesis into question. An association of saturated fat intake with CVD risk has not been consistently shown in prospective epidemiologic studies. Clinical trials that replaced saturated fat with n-6 polyunsaturated fat have mostly shown a reduction in CVD events, although several studies showed no effects. The effectiveness of replacement of saturated fat by monounsaturated fat has been called into question and replacement with a higher carbohydrate intake, notably those with a high glycaemic index, is considered inadvisable due to effects on insulin resistance and obesity.

Recent reviews have highlighted the complications in replacing saturated fat and trans-fatty acids intake with n-6 PUFA (linoleic acid) in controlled trials showed limited benefit and have suggested that n-3 PUFA may be responsible for the protective association between total PUFA and CVD. Low n-6 PUFA intakes are associated with increased uptake of dietary n-3 LCPUFA and may also enhance endogenous conversion of ALA to n-3 LCPUFA. Creating clear guidelines from the epidemiological and clinical trial data has been made increasingly difficult in an environment where total energy intakes have increased with the resulting global increase in obesity in all age groups.

Failure of fish oil supplementation in pregnancy to reduce the risk of gestational diabetes or pre-eclampsia

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Background: There is uncertainty regarding the efficacy of increasing n-3 long chain polyunsaturated fatty acid (LCPUFA) intake during pregnancy on reducing the risk of gestational diabetes mellitus (GDM) and pre-eclampsia (PE).

Objective: To determine whether n-3 LCPUFA supplementation in pregnancy reduces the incidence of GDM or PE. A secondary objective was to assess the effect of n-3 LCPUFA supplementation on perinatal complications.

Design: Double blind, multi-centre randomized controlled trial (RCT), the DOMInO Trial. Pregnant women (n=2399) under 21 weeks gestation were randomly assigned to receive docosahexaenoic acid (DHA) enriched fish oil (providing DHA 800 mg/d) or vegetable oil capsules without DHA, from trial entry to birth. The presence of GDM or PE was assessed through a blinded audit of medical records after birth. Birth outcomes and prenatal complications were also assessed.

Results: The overall incidence of GDM and PE was 8% and 5%, respectively, based on clinical diagnosis. The relative risk of GDM (RR 0.97, 95% CI: 0.74, 1.27) and PE (RR 0.87, 95% CI: 0.60, 1.25) did not differ between the groups. Birth weight, length and head circumference z-scores also did not differ between the groups. There were 12 perinatal deaths and 5 neonatal convulsions in the control group compared with 3 perinatal deaths and no neonatal convulsion in the DHA group (p=0.03 in both cases).

Conclusion: DHA supplementation of 800 mg/d in the second half of the pregnancy does not reduce the risk of GDM or PE. Whether supplementation reduces the risk of prenatal death and neonatal convulsions requires further investigation

Modulation of plasma fatty acid composition and conversion of 13C-α-linolenic acid to long-chain fatty acids by dietary high-oleic canola and flaxseed oils and genetic variants of FADS1 and FADS2

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The desaturation of dietary ALA to long-chain omega-3 PUFA is mediated through fatty acid desaturases (FADS1 and FADS2) and may be influenced by dietary fatty acid composition. The objective was to examine the effects of diets enriched in flaxseed oil (FXCO) or high-oleic canola oil (HOCO) versus a Western fat (WF) blend and single nucleotide polymorphisms (SNP) in FADS1 and FADS2 on plasma fatty acids, and 13C-ALA conversion and β-oxidation. Using a randomized crossover design, 36 hyperlipidemic subjects consumed 3 isoenergetic diets for 28 d enriched in FXCO (20.6 g/d ALA), HOCO (2.4 g/d ALA), or WF (1.3 g/d ALA). On day 27, blood was sampled at t = 0, 24, and 48 h after subjects consumed 45 mg of uniformly labelled 13C-ALA. Subjects were genotyped for rs174537, rs174545, rs174561, and rs174583 in the FADS1-FADS2 gene cluster. FXCO increased plasma ALA ~5-fold ($P < 0.001$), EPA ~3-fold ($P < 0.001$), and DPA by ~50% ($P < 0.001$), with no change in DHA compared with HOCO and WF diets. At 24 and 48 h the amount of administered 13C-ALA recovered as plasma 13C-EPA and 13C-DPA was lower ($P < 0.001$) after FXCO diet compared with HOCO and WF diets. No change in 13C-DHA was observed between diets. At 48 h post-dose, cumulative oxidation of 13C-ALA was similar (~19%; $P = 0.788$) between diets. Minor allele homozygotes of rs174537(TT), rs174583(TT), rs174545(GG), and rs174561(CC) had lower ($P < 0.05$) concentrations of plasma EPA, DPA, and lower ($P < 0.05$) 13C enrichment of plasma EPA at 24 and 48 h following all diets. In conclusion, although a high intake of ALA in FXCO diet increased plasma levels of n-3 fatty acids, this did not result in higher conversion efficiency, particularly for DHA. Furthermore, minor alleles of selected SNPs in the FADS1-FADS2 gene cluster are associated with reduce plasma fatty acid concentrations and conversion efficiency of 13C-ALA.

Optimized rapeseed oil naturally enriched with healthy micronutrients: a relevant nutritional approach to prevent cardiovascular diseases. Results of the Optim'Oils randomized intervention trial

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Rapeseeds are naturally rich in cardioprotective micronutrients but refining leads to substantial losses either by physical removal or by chemical reaction such as isomerisation. The Optim'Oils European project proposed innovative refining conditions to produce an optimized rapeseed oil enriched in micronutrients and low in trans linolenic acid. We aimed to investigate the cardioprotective properties of this Optimized oil. In a randomized, double-blind, controlled, cross-over study, 59 healthy normolipidaemic men consumed either the Optimized or a Standard rapeseed oils (20 g/d) and margarines (22 g/d) for 3 weeks. The Optimized oil reduced the trans FA concentration ($p = 0.009$) and increased the contents of alpha-tocopherol ($p = 0.022$) and coenzyme Q10 ($p < 0.001$) in comparison with the Standard oil. Over the 3 week trial, Total-/HDL-cholesterol and LDL-/HDL-cholesterol were increased by 4% ($p < 0.05$) with the Standard oil consumption whereas none of them rose with the Optimized rapeseed oil which increased the HDL-cholesterol and ApoA1 plasma content (+2%, NS and +3%, $p < 0.05$ respectively). The effects observed on the plasma HDL-cholesterol levels ($p = 0.059$), the Total-/HDL-cholesterol ratio ($p = 0.092$), and on the ApoA1 concentrations ($p = 0.060$) suggest an improvement of the cholesterol profile with the Optimized rapeseed oil. Finally, the Optimized oil reduced the plasma content of LDLox (-6%, NS), this effect being significantly different from the Standard oil ($p = 0.050$). In conclusion, reasonable intake of an Optimized rapeseed oil resulting from innovative refining processes and naturally enriched in cardioprotective micronutrients represent a relevant nutritional approach to prevent the risk of cardiovascular diseases by improving the cholesterol profile and reducing LDL oxidation.

The effect of omega-3 LCPUFA supplementation during pregnancy, or pregnancy and lactation, on infant cognitive and visual development: a systematic review and meta-analysis of randomised control trials

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Background: Maternal fish consumption during pregnancy has been positively associated with visual and cognitive abilities in the offspring, leading to the hypothesis that maternal omega-3 long chain polyunsaturated fatty acid (LCPUFA) supplementation may improve early childhood visual and neurological development.

Objective: The objective was to evaluate the effect of maternal omega-3 LCPUFA supplementation compared to a placebo in pregnancy on infant and child visual and neurological development.

Design: CENTRAL, MEDLINE, EMBASE, PubMed, PsychInfo and CINAHL databases as well as grey literature were searched for relevant articles. Human randomised control trials that supplemented the maternal diet with omega-3 LCPUFA during pregnancy or pregnancy and lactation and assessed either visual or neurodevelopment of the offspring were included. The quality of included trials was assessed with set criteria and results of eligible trials were compared in meta-analyses (when possible)

Results: No clear differences in standardized psychometric test scores were observed between groups. Mental development of LCPUFA-supplemented infants was 3.92 points higher than that of control children aged 2-5 (Mean difference (MD) 3.92; 95% CI 0.77 to 7.08; n=156; p=0.01), although this effect was from 2 trials with large attrition and high risk of bias. At no other age was there a difference between the groups in any psychometric measure of cognition although LCPUFA-supplemented infants had better eye and hand coordination (MD 6.00; 95% CI 1.03 to 10.97; n=72; p=0.02) compared to controls. Due to a variety of visual development assessments it was not possible to combine outcomes of different studies in a meta-analysis.

Conclusion: The evidence does not conclusively support or refute that omega-3 LCPUFA supplementation in pregnancy improves visual or cognitive development.

An Investigation into Polyunsaturated Essential Fatty Acids, Event Related Potential Assessments of Brain Function and Behavioural Measures in Children and Adolescents with and without Attention Deficit Hyperactivity Disorder

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Background: LC-PUFA's are implicated in neurodevelopment and abnormal in children with ADHD relative to controls. Trials with LC-PUFA have reported improvements in clinical symptoms of ADHD. However, the relationship between blood levels of LC-PUFA and clinical measures in ADHD is poorly understood and its relationship with neurocognitive functions in children with and without ADHD using event related potentials (ERPs) has not been investigated.

Objectives: To investigate:

1. Whether LC-PUFA blood samples differed between children with and without ADHD and relationships with clinical ADHD measures

2. A case-control comparison of EEG/ERP measures, and their relationship with fatty acids levels

3. The outcome of a 3 month PUFA supplementation trial on ERP neural activity in a subgroup of children with ADHD.

Participants & Procedure: 72 children were recruited. Three types of plasma were analysed, 9 behaviour questionnaires employed, and 3 ERP tasks were administered measuring motor/interference inhibition, sustained attention, and affect processing. The results of a separate RCT study with omega-3/6 fatty acids and the same ERP tasks are also reported in an ADHD subgroup (n=29).

Results: PUFA levels did not significantly differ between groups, but in ADHD, lower ω-3 was associated with greater ADHD and callous/unemotional traits symptom severity. In relation to the ERP measures there were no significant differences in ERP amplitude measures (P2, N2, P3). Significant relationships were observed between P3 ERP measures and PUFA fractions in ADHD and HC to task-relevant stimuli and EPA, DHA and total n-3 across frontal, central and parietal electrode sites. The supplementation ERP study, however, showed no significant effect of fatty acids intervention.

Conclusion: Although PUFA levels did not differ between groups, they were significantly associated with ADHD severity. Furthermore, significant relationships were observed between baseline levels of fatty acids and ERP components in both HC and the ADHD groups, suggesting the involvement of fatty acids in the neural activity of brain function.

A century of change in linoleic acid: Endocannabinoids, obesity and addiction

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

Breast cancer risk biomarkers are associated with dietary intake and tissue content of n-3 polyunsaturated fatty acids (PUFA)

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Background: Pre-clinical, observational, and case-control studies suggest that intake or tissue content of n-3 PUFA is associated with reduced risk of developing breast cancer.

Objective: The goal of this study was to examine relationships among diet, tissue fatty acid composition, and breast tissue biomarkers in women at increased risk of breast cancer from family history and/or previous biopsies.

Methods: Breast tissue was acquired by random periareolar fine needle aspiration from 74 women. Breast epithelial cells were assessed for cytomorphology and proliferation (Ki-67 immunochemistry), which are validated biomarkers for breast cancer risk. Fatty acid dietary intake was assessed with the National Cancer Institute Diet History Questionnaire. Fatty acid composition of erythrocyte, plasma, and breast phospholipids (PL) and plasma and breast triacylglycerols (TAG) were analyzed by gas liquid chromatography and expressed as wt% of total fatty acids.

Results: Overall dietary intake of n-3 PUFA was 1.1 ± 0.5 g/d, and the ratio of eicosapentaenoic acid (EPA)+ docosahexaenoic acid (DHA):arachidonic acid (AA) was 0.1:1.0 ± 0.09. Dietary intake of n-3

PUFA correlated with n-3 PUFA content as well as the ratio of DHA+EPA:AA in erythrocyte and plasma PL (r≥0.30; p≤0.017). Subjects with atypia consumed less n-3 PUFA: median of 0.84 vs 1.2 g/d (Mann-Whitney, p=0.020); and had lower total n-3 in plasma PL (4.4 vs 5.1%), plasma TAG (1.8 vs 2.3%), and erythrocyte PL (5.9 vs 7.1%) (p≤0.007). A lower ratio of breast TAG n-3:n-6 (0.053 vs 0.065) was associated with atypia (p=0.025). Cytologic atypia was most closely associated with erythrocyte PL DHA by logistic regression analysis (p=0.003).

Conclusions: Dietary intake of PUFA was reflected in tissue PL and TAG. Both lower n-3 intake and tissue content were associated with biomarkers of breast cancer risk.

Impact of n-3 supplementation on fatty acid composition of erythrocytes, plasma, and breast tissue in women at increased risk for breast cancer

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Background: In an observational study, we have found relationships between preneoplastic biomarkers in breast tissue and EPA and DHA content in phospholipid (PL) and triacylglycerol (TAG) fractions of erythrocytes and plasma. We hypothesized that EPA and DHA supplementation could reduce breast cancer risk biomarkers by increasing tissue n-3 PUFA; this study is ongoing. Here we report the effect of supplementation and withdrawal on PL and TAG composition of erythrocyte, plasma and breast.

Methods: Women (n=8 of proposed 60) at increased risk for breast cancer took LovazaTM (4 g/d; 1800 mg EPA and 1500 mg DHA) for 6 months in a single-arm study. We obtained blood at 0, 6, and 6.5 months and breast tissue before supplementation and at 6.5 months by random periareolar fine-needle aspiration. The fatty acid composition of erythrocytes, plasma, and breast tissue TAG and PL were analyzed by gas liquid chromatography and expressed as weight% of total fatty acids. Statistical analysis was performed using two-sided Wilcoxon signed rank test.

Results: Pre-study EPA, DHA, and AA in plasma TAG (0.20, 0.39, 1.71%, respectively) were about 4-fold higher than in breast TAG (0.04, 0.12, 0.39%). After 6 months of intervention with LovazaTM DHA content of erythrocyte PL increased from 3.0 to 5.4% (p=0.018), as did n-3:n-6 ratio of erythrocyte (0.19 to 0.49, p=0.018) and plasma (0.13 to 0.39, p=0.018) PL, and the n-3:n-6 ratio in breast (0.07 to 0.10, p=0.05) TAG. EPA+DHA:AA ratio increased in breast TAG (0.34 to 0.94, p=0.012).

Conclusion: LovazaTM increases the n-3 content in erythrocytes, plasma, and breast. Though breast tissue is much lower in long-chain PUFA compared to erythrocyte and plasma PL and TAG, supplementation increases n-3:n-6 and DHA+EPA:AA ratios in breast TAG.

Improved cognition in 4-year-old children who received dietary docosahexaenoic acid (DHA) and arachidonic acid (ARA) during their first 12 months of life

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BACKGROUND: Most commercial infant formulas sold worldwide as of 2007 contain DHA and ARA yet long-term cognitive outcomes remain controversial.

PURPOSE: To evaluate cognitive function in 4-yr-old children who received formulas containing DHA+ARA in the first year of life.

METHODS: Prior to 2005, newborn term infants (ages <9 d) were enrolled into 4 separate clinical trials and randomly assigned formula containing DHA (0.32-0.36%) and ARA (0.64-0.72%) or matched Control formula (no DHA or ARA) for 12 mo. Breast-fed (BF) infants were enrolled as a concurrent "gold standard". At 4 yrs of age, 142 children (50 DHA+ARA; 59 Controls; 33 BF) were assessed by Wechsler Preschool and Primary Scale of Intelligence (WPPSI-R or -III). DHA and ARA in red blood cells (RBCs) from the original trials were measured by GC/FID at 4 and 9 or 12 mo of age.

RESULTS: Mean±SD IQ scores in the DHA+ARA, Control and BF groups were Performance (109.8±12.5, 102.5±10.0, 108.7±14.2), Verbal (106.2±10.6, 97.8±10.9, 112.8±12.6) and Full-scale

(108.9±11.7, 100.3±10.4, 111.6±12.9). There was a main effect of diet group on all three IQ scores (all p<0.005). By Scheffé's pairwise comparison, the DHA+ARA group had higher Performance, Verbal and Full-scale IQs than Controls (all p<0.008). BF infants had higher Verbal and Full-scale IQs than Controls. Performance and Full-scale IQs were not different between BF and DHA+ARA groups, but Verbal IQ was significantly higher in BF than DHA+ARA infants (p=0.034). RBC-DHA at 4 & 9-12 mo was significantly correlated with Performance, Verbal, and Full-scale IQs of 4-yr-olds (Pearson r >0.31; p<0.002).

CONCLUSIONS: Cognition as measured by WPPSI IQ is superior in 4-yr-old children randomized to formula providing DHA+ARA for the first 12 mo of life compared to children not provided a supply of DHA and ARA during infancy, and was similar to that of breast-fed children.

Normalization of Whole Blood Viscosity in Cerebrovascular Accidents with Therapeutic Application of Phospholipid Emulsion

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Introduction: Our findings indicate clinical measurement of elevated whole blood viscosity to be clinical predictive of ischemic cerebrovascular accidents (CVA) and increased blood flow resistance in the cerebral microcirculation. Some preliminary research indicates interrelationships of blood brain barrier and endothelial dysfunction in other neurodegenerative diseases. In current study, we have measured normalization of whole blood viscosities in post-stroke patients using scanning capillary tube viscometer. Management and treatment protocols were determined by red cell lipid analysis of patients blood at Johns Hopkins, Peroxisomal Diseases Laboratory. Essential fatty acid EFA) restoration, EFA balance and removal of oxidized lipids were determined in preparations of oral and intravenous of phosphatidylcholine, glutathione, Subtilisin NAT, eicosapentaenoic acids and methylation factors to decrease whole blood viscosity, increase RBC deformability and initiate reperfusion of post-ischemic conditions resulting from CVAs.

Results: We have previously shown that the use of oral and IV lipids facilitated stabilization of decreased whole blood viscosity and restored microperfusion in several dozen subjects. This study suggests a correlation of whole blood viscosity and restoration of microcirculation with resolution of post-stroke neurological deficits. This WBV normalization corresponds to marked clinical improvement in our subjects within the first 3 months of intravenous lipid therapy.

Conclusions: We have documented significant clinical neurological improvement in our subjects along with marked normalization of RBC lipids and hemorheology (via laboratory analysis) following three months of an oral and intravenous lipid regime. The administration of our lipid protocol may offer a new therapeutic strategy for cerebrovascular accidents and monitoring whole blood viscosity in a study population with high risk factors and/or family history of CVAs may afford opportunity for early clinical intervention in prevention of CVAs and co-morbidity.

Why do cancer cells over-express fatty acid synthase?

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Background: Fatty acid synthase (FAS) is over-expressed in many human cancer cells even in the presence of pre-formed fatty acids supplied by the medium. Inhibition of FAS initiates apoptosis in cancer cells and decreases tumorigenesis in vivo, suggesting that FAS plays an essential role to cancer growth and survival.

Hypothesis: Cancer cells utilize endogenously synthesized fatty acids differently than those supplied exogenously, and so have a specific requirement for endogenously synthesized fatty acids.

Methods: Two human breast cancer cell lines, MCF7 and MDA-MB-231, and non-transformed MCF10A human breast epithelial cells were treated with C14 labeled acetate or palmitate. Total lipids were extracted from the cells and culture medium and radioisotope incorporation into total lipids, cellular lipid classes, phospholipid classes and cholesterol was measured using standard chromatographic techniques and liquid scintillation counting (LSC). Radio-labeled fatty acids were identified using high-performance liquid chromatography (HPLC) and LSC.

Results: No difference in incorporation of endogenously synthesized and exogenously supplied fatty acids into lipid or phospholipid classes was detected in any of the cell lines. HPLC revealed that endogenously synthesized and exogenously supplied fatty acids are primarily palmitate, palmitoleate and stearate. Analysis of the culture medium revealed that the cancer cells secrete endogenously synthesized fatty acids at 3 fold higher levels than non-cancer cells. Comparison of cancer and non-cancer cells revealed that cancer cells esterify proportionately less fatty acid to phospholipid and produce over 2 fold more choline glycerol phospholipid than non-cancer cells.

Conclusion: These results suggests that FAS over-expression does not fulfill a requirement for endogenously synthesized fatty acids in cancer cells as these fatty acids are not used for unique functions within the cell. In addition, a portion of endogenously synthesized fatty acids are excreted, suggesting that they are produced at higher levels than needed to support proliferation.

N-3 polyunsaturated fatty acids suppress phosphatidylinositol-(4,5)-bisphosphate dependent actin remodeling during CD4+ T cell activation

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N-3 polyunsaturated fatty acids (PUFA) [e.g., docosahexaenoic acid (DHA)] have been shown to exhibit anti-inflammatory properties; however, the mechanistic basis remains unclear. We have previously shown that n-3 PUFA attenuate events critical for T cell activation, including localization of F-actin to the immunological synapse (IS). Since the second messenger phosphatidylinositol-(4,5)-bisphosphate (PI(4,5)P2) resides in membrane lipid raft domains and DHA can alter the size of rafts, we hypothesized that PI(4,5)P2 and downstream F-actin remodeling are perturbed by the incorporation of n-3 PUFA into the plasma membrane, thereby suppressing T cell activation. We utilized the Fat-1 transgenic mice that expressed n-3 fatty acid desaturase from Caenorhabditis elegans, generating n-3 PUFA de novo and enriching the plasma membrane with n-3 PUFA. Additionally, conventional mice were fed either a 5% corn oil (CO, control) or a 4% DHA triglyceride-enriched diet. Splenic CD4+ T cells from Fat-1 mice exhibited a 50% decrease in PI(4,5)P2 as determined by mass spectrometry. Similarly, CD4+ T cells from mice fed a DHA-enriched diet exhibited a 25% decrease in PI(4,5)P2. Upon activation by anti-CD3/anti-CD28 or PMA/ionomycin, wild type (WT) and CO-fed CD4+ T cell PI(4,5)P2 levels decreased 25-50% within 5 min, whereas PI(4,5)P2 remained unchanged in Fat-1 and DHA-enriched CD4+ T cells. Furthermore, actin remodeling, assessed by immunofluorescence, was significantly increased in WT and CO-fed but not in Fat-1 and DHA-enriched CD4+ T cells upon activation. The Wiskott-Aldrich syndrome protein (WASP), an actin-remodeling protein regulated by PI(4,5)P2, was recruited to the IS upon anti-CD3/anti-CD28 coated bead stimulation in WT, but not Fat-1 CD4+ T cells. The defect in actin remodeling in Fat-1 CD4+ T cells was rescued by incubation with exogenous PI(4,5)P2 in a dose-dependent manner. These data demonstrate that DHA alters PI(4,5)P2 in CD4+ T cells, thereby suppressing actin remodeling and downstream events critical for T cell activation.

Impact of human milk and structured lipids on lipoprotein lipids in infants

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The mammary gland has unusual pathways of lipid synthesis that result in truncation of fatty acid synthesis at a carbon chain length of 14 or lower, and uptake of palmitic acid (16:0) from maternal plasma to maintain a relative proportion of 16:0 in human milk fatty acids, regardless of the maternal diet. Triglyceride (TG) synthesis is also unusual, and involves preferential positioning of 16:0 at the sn-2 (centre) carbon of the TG glycerol backbone, with 18:1n-9, 18:2n-6,18:3n-3 and medium chain fatty acids directed towards the TG glycerol sn-1,3 positions. Much of the interest in milk TG structures has focused on intraluminal events. However, retention of the unusual milk TG structure post-absorption may influence

fatty acid delivery to developing tissues, and hence metabolic consequence. Plasma lipoprotein lipids were compared between infants randomized to be fed formula containing structured TG enriched in 2-palmitate or 16:0 from palm olein, and to breast-fed infants at 60 and 120 days of age. Parallel studies enabling access to tissue lipids were done in piglets fed similar formula. The chylomicron TG of breast-fed infants and infants fed formula with sn-2-palmitate showed retention of sn-2 palmitate TG species. Higher 16:0, but lower 18:1n-9 and 18:2n-6 in plasma monoglycerides of breast-fed infants suggests the milk TG structure may influence tissue fatty acid delivery, with preferential delivery of unsaturated fatty acids to extra-hepatic tissues, with retention of saturated monoglyceride products of lipoprotein lipase. The TG structure and fatty acid composition also impacted the liver TAG and cholesterol, and tissue fatty acids in animals. The analyses of lipoprotein lipid, monoglyceride and fatty acids in plasma indicates that human milk TAG may be specifically organized for targeted delivery of not only which but how fatty acids are delivered to developing infant tissues.

Postprandial lipaemia, APOE genotype and responsiveness to dietary fat manipulation

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With the pattern of meal ingestion in Western Societies, individuals spend the majority of the day in the postprandial state. In recent years, non-fasting triacylglycerol (TAG) concentrations have emerged as a clinically significant cardiovascular disease (CVD) risk factor, with hazard ratios of 2-4 in the highest versus the lowest levels of non-fasting TAG. Given the strength of these associations are comparable to those of low density lipoprotein cholesterol (an established CVD risk factor); there is considerable interest in understanding the independence of the association, and causality of TAG in CVD. The postprandial TAG response has been shown to be highly variable between individuals, with the apolipoprotein (APO)E (epsilon) genotype likely to be an important genetic determinant as a result of the significant role apoE plays in lipoprotein metabolism. However, the majority of information regarding the effects of dietary fats on lipid levels in this genotype group has been derived from studies performed in the fasting state. Although findings are inconsistent, there is some suggestion that APOE4 carriers may show a greater responsiveness of fasting lipids to dietary fat manipulation compared with E3/E3 individuals. Since both the amount and type of fat given in a meal has been shown to influence the magnitude and duration of the postprandial lipaemic response, little is known about the independent and interactive impact of APOE genotype. An overview of the relationship between non-fasting TAG, dietary fat and APOE genotype will be presented; together with recent findings from our group which suggest that dietary fat manipulation may be a more important modulator of the subsequent postprandial TAG response than APOE genotype.

Ruminant trans-11 vaccenic acid decreases fasting and post-prandial hypertriglyceridemia by reducing both hepatic and intestinal TG secretion and associated genes in a rat model of the metabolic syndrome

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Background: Ruminant trans-11 vaccenic acid (VA) is the predominant naturally occurring trans fat in the food chain. Both fasting and non-fasting (post-prandial) hypertriglyceridemia are risk factors for cardiovascular disease (CVD) and VA has been shown to decrease fasting hypertriglyceridemia in several pre-clinical animal models. However, the mechanistic action by which VA exerts its hypotriglyceridemic benefits remains unclear. The objective of this study was i) to evaluate the effects of VA on both intestinal (fed state) and hepatic (fasting state) TG secretion and ii) to profile intestinal and hepatic gene expression using the JCR:LA-cp, a rat model of the Metabolic Syndrome (MetS).

Methods and Results: MetS rats were assigned to a control diet (CD) with or without 1% w/w VA. Hepatic TG secretion was assessed during fasting conditions whilst intestinal TG secretion was assessed after an oral gavage of [3H] triolein in olive oil following lipoprotein lipase inhibition. An array of genes (n=44) involved in lipid synthesis, oxidation and transport was performed by real time-PCR using a quantitative 'high-throughput' system. VA reduced hepatic TG secretion relative to lean, but not MetS control rats

(p>0.05); whilst VA reduced intestinal TG secretion (24%) relative to MetS control rats (p<0.05). The reduction in TG secretion by VA was concomitant with decreased intestinal and hepatic TG tissue mass (-43% and -64% versus control). From all 44 genes measured, VA was observed to down-regulate intestinal SREBP1 and FAS, whilst up-regulating hepatic DGAT2, AGPAT2, HMGCoA-R, APOA1and SRB1 mRNA.

Conclusion: VA can improve fasting and post-prandial hypertriglyceridemia in the JCR:LA-cp rat by reducing intestinal and hepatic TG secretion. Gene expression data suggests opposing transcriptional control in the liver and intestine as a consequence of VA supplementation. We propose that VA supplementation may contribute to improving fasting and post-prandial hypertriglyceridemia during conditions of MetS and reduce CVD risk.

Epidemiologic studies on the intake of ruminant trans fatty acids and the risk of CVD

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Observational epidemiologic studies have shown a higher risk of coronary heart disease (CHD) to be associated with the intake of trans fatty acids (Mozaffarian et al., 2009). Trans fatty acids arise either from industrial hydrogenation of vegetable oils, or from hydrogenation of unsaturated fatty acids in the rumens of ruminants. As a result of biohydrogenation, meats and dairy products from cows, sheep, and other ruminants contain trans fatty acids. The sources of industrially produced trans fatty acids are fast foods, bakery products, package snack foods, margarines, and crackers. Overall, the evidence from observational studies suggests that higher CHD risk is related to the intake of trans fatty acids from industrial sources rather than trans fatty acids from ruminant sources (Mozaffarian et al., 2009). Observational studies on the intake of trans fatty acids from ruminant sources and the risk of CHD were reviewed.

The association between the intake of trans fatty acids from ruminant sources and the risk of CHD has been investigated in several observational studies. Among the case-control and follow-up studies, one study (Pietinen et al., 1997) showed a significant negative association, where five other studies (Willett et al., 1993; Ascherio et al., 1994; Oomen et al., 2001; Jakobsen et al., 2008; Laake et al., 2011) found no statistically significant associations. In a cross-sectional study (Bolton-Smith et al., 1996), the intake of trans fatty acids from ruminant sources was significantly associated with a lower risk of CHD among men, but not among women.

In summary, observational studies suggest that the intake of trans fatty acids from ruminant sources is not associated with the risk of CHD within the range of intake in the general population. However, these data do not exclude the possibility that higher intakes than the amounts actually consumed could have adverse effects.

Red blood cell omega-3 fatty acid levels and neurocognitive performance in deployed U.S. Servicemembers

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Objective: To explore the cross sectional relationships between blood EPA+DHA (HS-Omega-3 Index®) and sleep disorders, depression, anxiety, and neurocognitive performance in Servicemembers deployed to Iraq.

Methods: Servicemembers with mild to moderate depression by the Patient Health Questionnarie-9 from two US military camps were invited to participate in this study. A battery of validated psychosocial (Pittsburgh Sleep Quality Index, and Zung Depression, Zung Anxiety, Espworth Sleepiness, and Combat Experiences scales) and computerized neurocognitive tests were completed by each participant. Five neurocognitive domain scores were calculated - Processing Speed, Complex Attention, Reaction Time, Cognitive Flexibility (CF), and Executive Function (EF). A drop of blood was also collected on an anti-oxidant-treated filter paper card and sent for HS-Omega-3 Index analysis. An ANOVA contrast was used to test for linear trends between quartiles of the HS-Omega-3 Index for both EF and CF.

Results: The mean HS-Omega-3 Index was $3.5 \pm 0.7\%$ (n=78). The HS-Omega-3 Index was not significantly associated with scores for anxiety, depression, or sleep, whether assessed as continuous or dichotomous variables, but was directly associated with CF and EF ($p<0.02$ and 0.01, respectively), especially in the 81% who reported poor sleep quality. In those with poor sleep quality (n=63), EF and CF were higher ($p=.005$) in subjects with Omega-3 levels above versus below the mean.

Conclusion: Optimal neurocognitive performance is essential during deployment. Our finding that EF and CF were positively related to HS-Omega-3 Index suggests that improving omega-3 status through an increase in omega-3 intake may improve neurocognitive performance and confer an element of resilience to poor sleep.

Monounsaturated and high linolenic acid oils, plasma lipids and fatty acid metabolism

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Considerable interest has focused on the influence of dietary fat quality on cardiovascular disease (CVD) risk. Particularly, novel dietary oils rich in oleic acid and α-linolenic acid (ALA) are being developed and marketed with an aim to improve fatty acid intakes and reduce CVD risk. Our objective was to investigate the efficacy of high-oleic canola oil (HOCO) alone, or blended with flaxseed oil (FXCO), on traditional and emerging clinical biomarkers of CVD risk. A further goal was to study the influence of dietary factors on metabolism of 13C-ALA to long-chain PUFA. Using a diet-controlled randomized crossover design, thirty-six hypercholesterolaemic subjects consumed three isoenergetic diets for 28 days each containing ~36% energy from fat, of which 70% was provided by HOCO, FXCO, or a Western diet (WD; control). Endpoint measures revealed reductions in serum lipid concentrations, including 7.4% and 15.1% decreases in LDL-cholesterol concentrations after the HOCO and FXCO diets, respectively, as compared with the WD control. Moreover, reduced plasma E-selectin concentrations were found after the FXCO diet compared with the WD control. Consumption of the dietary oils failed to alter whole-body fat oxidation or energy expenditure, nor lead to any changes in body composition. FXCO diet increased plasma ALA ~5-fold, EPA ~3-fold, and DPA by ~1.5-fold, but failed to alter DHA levels compared with the WD control. Up to 48 h, conversion rates of 13C-ALA to plasma 13C-EPA and 13C-DPA were lower after FXCO diet compared with HOCO and WD diets, suggesting decreased ALA transmission to long chain PUFA with very high intakes of dietary ALA. No differences in plasma 13C-DHA enrichment were observed across diets. Taken together, substitution of dietary fats common to WD with both HOCO and FXCO represents a useful approach to simultaneously target several biomarkers for CVD risk reduction.

Disturbance Of Phospholipid Membrane Structure In Neurological Disease

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The lipid soluble nature of toxins has led us to address the complexity of neurotoxicity oriented to cell membrane architecture. Identification of nuclear and mitochondrial DNA adducts as microbials, chemicals, pesticides, and metals at Acumen Laboratory in Devon, England and red cell lipid analysis at Johns Hopkins, Peroxisomal Diseases Laboratory were obtained on subjects with Multiple Sclerosis, Autism, Post Stroke, Peripheral Neuropathy, epilepsy, neurometabolic disorders, Alzheimers, Motor Neuron, and Parkinsons Disease. Our previous findings revealed a link between toxic exposures, a characteristic accumulation of very long chain fatty acids (VLCFAs) in the form of lipid rafts or ceramides, and the development of cell membrane derangement resulting in dysfunction. In our current study we have captured visual images of toxins on the cell surface which have caused disturbances in cellular phospholipid structure and mitochondria of individual subjects, and have linked the impact of the DNA adducts (toxins) altering gene expression to aberrations in lipid metabolism, cellular dysfunction and alteration of the structure of phospholipids in the cell membrane characteristic to the presenting diagnosis and symptoms. In this uncontrolled patient group, our treatment protocol of intravenous infusion of phosphatidyl choline to clear bioaccumulation of toxins and stabilization of cellular structures has yielded marked clinical neurological improvement in our 150 subjects corresponding with significant improvement in red cell lipid analysis and apparent normalization of cellular structure and function based on images of

the subjects membrane phospholipids. While further work remains and the microscopic significant of these correlations needs further clarification, initial biochemical and clinical results appear promising.

Psychophysiological effects of Krill oil: A double blind clinical trial

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Krill oil is obtained from Antarctic krill (Euphausia superba Dana). Krill oil contains about 45% phsopholipids, of which 95% is phosphotidyl choline. The phospholipids in Krill oil are rich in EPA and DHA. Phospholipid bound LC-PUFAs are better transported to brain. Radial maie study in rats confirmed neuroprotective and memory boosting effects of krill oil. In this study we expanded the work in human to study if the krill oil adminstration will improve mental perfomance.

Using double blind, randomized, placebo control trial, 45 adult Japanese men (62 - 75 years) were divided into 3 groups; fish oil group, krill oil group and palcebo group. The subjects were given 2 g of the test substance in capsule form for 12 weeks. At the end of 12 week intervention, the subjects were subjected to brain functional neuroimaging to measure 1) oxyhemoglobin concentration by near infrared spectroscopy (NIRS) when the subjects performed a memory task and a calcualtion task and 2) brain event related potential P-300.

None of the interventions showed any side effects. Krill oil treatment increased the oxyhemoglobin concentration in anterior and temporal areas of the brain following memory and calculation tasks. The increase in oxyhemoglobin concentration was much higher with krill oil than with fish oil or placebo treatment. Depending on the task, the different areas of the brain showed increase in oxyhemoglobin concentrations, suggesting different parts of brain are involved in different activities. P300 measurement is an index of overall activity of brain. The amplitudes of P300 wave is related to cognitive funciton while the latency of the wave is associated with the speed of information processing. Kril oil treatment reduced the latency, indicating it improved the speed of information processing in adult males.

Serum level of arachidonic acid, EPA and DHA and risk of cognitive decline: cross-sectional analysis of Japanese elderly in National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA)

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Background: The number of elderly patients with dementia is increasing in Japan, and the lower level of LC-PUFA in the brain and plasma of dementia patients has been reported.

Objective: To determine whether serum level of LC-PUFA (arachidonic acid (ARA), EPA and DHA) is associated with the risk of cognitive decline in Japanese elderly.

Methods: This cross-sectional study included 576 men and 584 women aged 60 to 88 years who participated in the fifth wave examination (2006-08) of the NILS-LSA (National Institute for Longevity Sciences-Longitudinal Study of Aging) in Japan. Venous blood was collected early in the morning after at least 12 h fasting and serum was stored at -80°C until assay. Serum fatty acid composition was measured by gas-liquid chromatography. Cognitive decline was defined as Mini-Mental State Examination score (0-30) <= 27. The lowest quintile category (Q1) of the fatty acid was used as a reference, and the effects of each quintile category for fatty acid on cognitive decline was estimated by multiple logistic regression models controlled for age, sex and education (<10y, 10-12y, 12y< of school).

Results: 248 men (43%) and 205 women (35%) classified cognitive decline. Mean (± SD) serum level of ARA, EPA and DHA (% of total fatty acids) in 1,160 participants was 5.4 (± 1.1), 2.8 (± 1.4), and 6.0 (± 1.4) %, respectively. The odds ratio of cognitive decline across each quintiles (Q2-Q5) of PUFA level was ranged from 0.67 to 0.88 in ARA, 0.65 to 0.86 in EPA, and 0.83 to 0.90 in DHA, and statistically

significant in ARA (odds ratio of the Q3 to Q1: 0.67, 95%CI: 0.46-0.99) and EPA (odds ratio of the Q4 to Q1: 0.65, 95%CI: 0.44-0.96).

Conclusion: These results suggest that low serum concentration of ARA and/or EPA may be associated with a risk of cognitive decline in Japanese elderly.

Measurement of Protein Palmitoylation by Advanced Mass Spectrometry

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Palmitoylation or S-acylation of proteins is a reversible and versatile post-translational protein modification (PTM), which involves a covalent binding of a fatty acid (mostly palmitic acid) to free thiols of cysteine residues. This PTM plays important functional roles in cellular processes, such as membrane anchoring of protein (complexes), signaling, trafficking and protein-protein interactions.

Measurement of palmitoylation is challenging due to a lack commercially available standards, poor long-term stability of the palmitic thioester bond and lack of adequate enrichment- or sensitive enough methods for detection. Traditional methods to detect protein palmitoylation or S-acylation are laborious, involve radioactive and/or other labeling methods and are potentially not sensitive enough for the detection of novel protein palmitoylation sites.

In recent collaborative efforts, we have shown that direct mass spectrometric analysis of S-acylated peptides labeled with palmitate or palmitate analogues, obtained through enzymatic digestion of palmitoylated proteins is readily possible. Furthermore, specific release of palmitate from S-acylated sites and relabeling with stable-isotope labeled alkylating agents, such as for example deuterated N-ethylmaleimide, allows for quantitative comparisons of palmitoylation extent between samples of different origin, e.g. proteins from organisms exposed to different fatty acid contents via different diets. Measurements are achieved employing matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), after enzymatic protein digestion and enrichment of palmitoylated peptides. In combination with mutation experiments and our MALDI-MS approach we discovered a novel transacylation mechanism in 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS2), the rate limiting enzyme in ketogenesis. Here, we will present further examples and an overview of our new approaches towards characterisation and measurement of S-acylated proteins, employing advanced mass spectrometry.

Effects of DHA Supplementation on Lipocentric and Glucocentric Markers of Insulin Resistance in Hypertriglyceridemic Men

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Increase in obesity and metabolic syndrome are associated with increases in insulin resistance (IR) and type 2 diabetes mellitus. Results from human epidemiological studies suggest that n-3 PUFA can prevent IR. A number of cross-sectional human studies reported negative associations between IR and tissue concentrations of n-3 PUFA, but results from human intervention studies have varied. Besides the changes in glucocentric markers of IR, an equally important aspect of IR is the effects of insulin on lipid metabolism (lipocentric markers). Several physiological markers of lipid metabolism, such as receptor for oxidized LDL (OLR1), ratio between plasma triglycerides (TG) and HDL-C, number of small dense (sd) LDL particles, non-esterified fatty acids (NEFA), are positively associated with IR. By using a placebo controlled, parallel study design, we examined the effects of DHA supplementation (3 g/d, 90 d) in the absence of EPA on glucocentric and lipocentric markers of IR in hypertriglyceridemic men (n=14-17/group). DHA supplementation increased fasting plasma glucose concentration by 4.7%, ($p<0.05$) but that did not differ from the 2.7% increase in the placebo group. It also did not alter other indices of IR based on fasting (insulin and HOMA-IR) or postprandial insulin and glucose concentrations (areas under curves for insulin and glucose, Matsuda index). DHA decreased expression of OLR1on white blood cells by greater than 60 % as tested by microarray gene chip analysis and confirmed by qRT-PCR. It also

decreased circulating concentrations of NEFA (13 %), small dense LDL particles (22 %), and TG:HDL-C ratio (34 %) ($p<0.05$). None of the variables changed in the placebo group. Our results suggest that lipocentric markers of IR are more responsive to DHA supplementation than the glucocentric markers. Future studies with DHA in pre-diabetic subjects and direct measures of insulin sensitivity are needed.

Molecular Mechanisms for Docosahexaenoic Acid-Derived Neuroprotection

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Enrichment of polyunsaturated fatty acids, especially docosahexaenoic acid (DHA, 22:6n-3), in the brain is known to be critical for optimal brain development and function. As DHA is readily incorporated into the phospholipids in neuronal membranes, DHA can influence not only chemical and physical properties of cell membranes but also membrane related signaling events involved in neuronal survival, proliferation and differentiation. Our studies have indicated that DHA supplementation increases phosphatidylserine (PS) accumulation and inhibits neuronal cell death under challenged conditions, supporting a notion that DHA is an important neuro-protective agent. We also found that DHA released from the membrane is metabolized to a potent synaptogenic agent, synaptamide (N-docosahexaenoylethanolamide), promoting neurite growth, synaptogenesis and glutamatergic synaptic function in developing neurons as well as neuronal differentiation of neural stem cells (NSCs). Molecular and signaling mechanisms underlying DHA-mediated beneficial effects will be discussed in the context of neuroprotection, particularly under challenged conditions such as in brain injury.

Physical properties of lipid droplets in the early diet affect adipose tissue development in C57Bl/6j mice

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We previously showed a sustained effect of an increase in n3 PUFA in the postnatal diet on fat mass accumulation in adult mice (Oosting et al., Pediatr. Res., 2010, 68(6), 494-9). Besides composition, fat quality also encompasses physical properties of lipid droplets. Compared to current infant formula, lipid globules in human milk are up to 10 times larger and coated by a phospholipid membrane. We developed a concept with a complex lipid matrix (Nuturis®) more closely resembling these lipid properties and investigated long-term effects on body composition- and adipose tissue development in adulthood.

Male mice received a diet containing either Nuturis® or standard formula (CTRL) from postnatal day (PN) 16 to 42. Subsequently, mice were challenged with a moderate Western style diet (WSD; 20% w/w fat, 0.1% w/w cholesterol) until dissection at PN98. Body composition was monitored by DEXA-scan throughout the WSD challenge. Weight, adipocyte number, size distribution and gene expression were analysed in white adipose tissue (WAT) depots.

At PN98, body weight, fat mass and individual WAT depot weight were lower for Nuturis® compared to CTRL mice. Lean body mass, food intake and adipocyte number were similar in all groups. Compared to CTRL mice, Nuturis® mice had a shift in the adipocyte size distribution, resulting in a higher percentage of small adipocytes. Also, gene expression of adipocyte size markers (leptin, Mest/Peg1), was lower in Nuturis® compared to CTRL mice. The expression of Pref1 (preadipocyte marker) remained unaffected by any of the diets, illustrating comparable preadipocyte numbers.

This study shows that a postnatal diet containing a complex lipid matrix (Nuturis®), results in reduced fat accumulation and smaller adipocytes in an obesogenic adult environment, emphasising the contribution of physical properties of lipid droplets to body composition development later in life. .

1 Oosting et al., Pediatr. Res., 2010, 68(6), 494-9.

Omega-3 LC-PUFA supply and neurological outcomes in children with phenylketonuria (PKU)

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Children with phenylketonuria (PKU) follow a diet with very low intakes of natural protein which is devoid of food sources of the omega-3 docosahexaenoic acid (DHA). A resulting DHA depletion has been demonstrated in PKU children and may account for detectable subtle neurological deficits that are not explained by variation in plasma phenylalanine concentrations. We supplemented 36 children with PKU aged 1 to 11 years for 3 months with encapsulated fish oil providing a daily dose of 15 mg DHA/kg body weight. DHA supplementation resulted in significantly faster visual evoked potential latencies, indicating more rapid central nervous system information processing. In addition, DHA significantly improved outcomes in a standardized test of motor function and coordination. No changes over time were seen in aged matched healthy controls. Since the PKU children had a good supply of the omega-3 precursor alpha-linolenic acid, these observations lead us to conclude that endogenous conversion of alpha-linolenic acid is not sufficient to provide adequate amounts of DHA that support optimal function. Hence DHA appears to be a conditional essential substrate for children with PKU. Since early treated PKU children are healthy, with normal fatty acid turnover, these data may indicate a need to supply some DHA to children in general. In an ongoing multicentric study funded by the European Commission, we aim at establishing quantitative dose response between DHA supply and functional outcomes that should help defining quantitative DHA requirements in children.

Polyunsaturated fatty acids and child health: diet or genes?

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An adequate provision of polyunsaturated fatty acids (PUFA) has long been considered essential for supporting child health, including with regards to neuronal development and occurrence ofallergic diseases. In the last few years, genetic association studies demonstrated that in addition to nutritional inatkes, the genetic background is highly important for PUFA composition of human blood and tissue lipids. Specifically, polymorphisms in the fatty acid desaturase (FADS) gene cluster determine the efficiency how PUFAs are processed endogenously. Recent gene-nutrition interaction studies suggest that these polymorphisms modulate the effect of dietary fatty acid intake on complex phenotypes such as cognitive outcomes and asthma risk in children. These first results may provide the basis for future, more specific intake recommendations to achieve optimal health benefits for all children. We present results from those recent gene-nutrition interaction studies, discuss the implications for future observational and intervention studies as well as for child health, and provide suggestions as to how this association might translate into clinical practice in the future.

Omega 3 fatty acids and metabolic syndrome

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Obesity and associated diseases, namely type 2 diabetes, dyslipidaemia and hypertension, i.e. components of the metabolic syndrome, represent a major threat for the health care systems in affluent societies. Complex etiology of metabolic syndrome implies the need of treatments, which are based on multiple mechanisms of action. Development of the syndrome could be delayed by lifestyle modifications, while both dietary and pharmacological interventions are required for the therapy. Naturally occurring n-3 long-chain PUFA, namely eicosapentaenoic and docosahexaenoic acids (Omega-3), exert pronounced anti-inflammatory effects, act as hypolipidaemics, reduce cardiac events and may decrease the progression of atherosclerosis. However, Omega-3 fail to improve glycaemic control in diabetic patients.

Experiments in mice fed high-fat diet revealed that Omega-3 could prevent development of obesity and hepatic steatosis, while modulating liver, adipose tissue, intestine and muscle metabolism. These effects of Omega-3 reflect changes in fatty acid composition of phospholipids, formation of Omega-3-derived lipid mediators, gene expression, as well as increases in the activity of adiponectin-AMPK axis and decrease

in the activity of endocannabinoid system. Importantly, Omega-3 could augment beneficial effects of other treatments. Thus, (i) a combination treatment using Omega-3 and a mild calorie restriction efficiently reduced body fat accumulation, while inducing a metabolic switch toward lipid catabolism in adipose tissue; and (ii) a combination with anti-diabetic drugs thiazolidinediones exerted additive effects in the amelioration of dyslipidaemia and insulin resistance, while preserving muscle insulin sensitivity and metabolic flexibility, and reverting insulin resistance. Both combination treatments strongly suppressed low-grade inflammation of adipose tissue. Combination treatment using Omega-3 and a low dose of rosiglitazone reduced obesity. These results are relevant for the prevention and therapy of obesity and its comorbiditiesatment

Effect of pomegranate seed oil on the lipid matabolism in Wistar rat

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CLnA is one of the highly unsaturated forms of conjugated fatty acids with triple bonds that occurs in multiple positional and geometric isomers (cis and trans) of linolenic acid (LnA, cis-9,cis-12,cis-15 C18:3n-3). CLnA has been found abundantly in some seed oils, such as Pomegranate seed oil (cis-9,trans-11,cis-13; C18:3).

The objective of this study was to evaluate the effect of Pomegranate seed oil as a source of CLnA compared to CLA (cis-9,trans-11) on lipid metabolism in Wistar rats.

Twenty four Wistar rats were randomly assigned to four experimental groups and fed for the next four weeks. The experimental diets were: I – Control (AIN-93G), II – Flaxseed oil (as a source of LnA), III – Pomegranate seed oil (as a source of CLnA) and IV – CLA (cis-9,trans-11). Experimental diets were supplied with seed oils equivalent to an amount of 1% of studied fatty acids. Plasma samples were analyzed using kits for total cholesterol (TC), triacylgycerols (TAG) and HDL cholesterol. LDL+VLDL cholesterol level was calculated. Tissue lipid profile (FAME, Varian CHROMPAK – 3380) and SCD-1 and FAS gene expression (Real-Time PCR) were analysed.

The experimental treatments had no effect on plasma TC in rats. At the same time, the LDL+VLDL cholesterol and TAG were significantly decreased in animals fed Flaxseed oil, Pomegranate seed oil and CLA compared to Control (LDL+VLDL: 0.4, 0.4, 0.5, respectively vs 0.9mmol/L; TAG: 1.6, 1.8, 2.2, respectively vs 3.0mmol/L). Additionally, Flaxseed oil significantly increased HDL cholesterol level compared to Control group (1.7 vs 1.1mmol/L). Additionally, the fatty acid composition in rats fed Flaxseed oil and Pomegranate seed oil was significantly changed. In the same line, stearoyl coenzyme A desaturase (SCD-1) index was changed. In rats fed Pomegranate seed oil liver's FAS gene expression tended to decreased.

In conclusion, Pomegranate seed oil improved the lipid metabolism more effectively than CLA in laboratory rats.

Nuts Decrease Chronic Disease Risk via Multiple Mechanisms

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Epidemiologic studies consistently demonstrate a beneficial association of increased nut and peanut consumption with coronary heart disease and sudden cardiac death. In addition, nut consumption is inversely associated with hypertension, type 2 diabetes and metabolic syndrome. A recent study using NHANES 1999 to 2004 data with 13,292 adults (≥ 19 years of age) reported decreased body weight, BMI, waist circumference and systolic blood pressure in nut consumers versus non-nut consumers. In addition, nut consumers had fewer criteria for metabolic syndrome. C-reactive protein also was lower in nut consumers. Numerous clinical studies have provided insight about the underlying mechanisms that account for the health benefits of nuts. A pooled analysis of 25 nut consumption trials (n=583 men and women) demonstrated dose-dependent reductions in total cholesterol (TC), LDL-cholesterol (LDL-C), and the TC/LDL-C ratio. Plasma triglycerides decreased in hypertriglyceridemic individuals. Nuts also have beneficial effects on an array of new CVD risk biomarkers such as LDL oxidizability, soluble inflammatory

molecules, and in individuals with hypercholesterolemia and type 2 diabetes, walnuts improve endothelial function. There is emerging evidence that pistachios favorably affect LDL and HDL particle size. In addition, there is evidence that postprandial walnut consumption may enhance HDL particle functionality and promote reverse cholesterol transport. With respect to body weight, potential explanatory mechanisms include satiety value of nuts, induction of energy expenditure and inefficient energy absorption. Animal studies have reported beneficial effects of walnuts on reduction in breast and colorectal cancer, as well as cognitive function. In addition to the favorable fatty acid profile of nuts and peanuts, other bioactive compounds that explain their health benefits include: macronutrients including plant protein and fiber; micronutrients including potassium, calcium, magnesium, and tocopherols; and phytochemicals such as phytosterols, phenolic compounds, resveratrol, and arginine.

Providing a Novel Lipid Emulsion Containing N-3 Fatty Acids Decreases Procalcitonin and Lymphocyte Concentration in Infants after Cardiac Surgery

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Introduction: The effect of parenteral n-3 fatty acids in critically ill infants, who are at risk of infection, is not widely studied. Plasma concentrations of procalcitonin (PCT) and lymphocytes (LYMPH) are biomarkers of infection. This study investigated the effects of parenteral fish oil on plasma lymphocyte and procalcitonin concentrations.

Methods: Infants (n=33) undergoing cardiopulmonary bypass for open-heart surgery, and in need of parenteral nutrition were randomized to receive either soybean oil (control) or a 50:40:10 mixtures of medium-chain triglycerides (MCT), soybean oil and fish oil (n-3 treatment). Parenteral nutrition was administered continuously for 3 days pre-operatively and 10 days post operatively. PCT and LYMPH were quantified in plasma at baseline, before surgery and on days 1, 7 and 10 after surgery .Pediatric risk of mortality (PRISM) scores were recorded on admission to the pediatric intensive care unit.

Results: The plasma PCT between sampling periods was statistically significantly different in both study groups (p=0.0001).The PCT was significantly higher in the control versus the n-3 treatment group 24 hours after open heart surgery (p=0.01) but not at the other time points. In a post-hoc subgroup analysis, on post operative day 10 LYMPH in infants with low (n=17) vs. high (n= 16) PRISM scores was significantly different (p=0.0008).In those with high PRISM scores, the n-3 treatment group (n=9) exhibited a 45 % lower LYMPH than the control group (n=7).

Conclusions: Inclusion of a lipid emulsion containing MCT and n-3 fatty acids in parenteral nutrition provided to critically ill infants may decrease plasma PCT and LYMPH concentration postoperative cardiac surgery. This observation suggests that an n-3 lipid emulsion containing 20:5n:3 and 22:6n:3 may suppress the inflammatory response induced by cardiopulmonary bypass and surgery, and therefore may be beneficial to critically ill infants undergoing heart surgery.

Could an apparently inconsistent relationship between n-3LCPUFA and blood pressure in children be explained by changes in anxiety?

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The long-chain n-3 fatty acid (n-3LCPUFA) research has focused on cardiovascular benefits in adults and brain development in infants, whereas potential effects in childhood has been given little attention. My group has investigated the effect of n-3LCPUFA-supplementation (~1g/day) on blood pressure in late infancy as well as in teenage boys and found that this decreased after three and four months, respectively, which is unexpected based on results in healthy adults. We have recently made two observational studies in order to see if such an association was detected within children. However, in both 17 years-old children from the Copenhagen Birth Cohort study and in a cross-sectional study of 9-11 years-old school children we found significantly higher blood pressure in those with high n-3LCPUFA-levels in their erythrocytes or whole blood. This was in agreement with results from our 7-year follow-up study in children, whose mothers had been randomized to fish oil as compared to those whose mothers

had been given olive oil during the first four month of lactation. Since we a priori assume one common mechanism behind the effect of n-3LCPUFA on blood pressure, we speculate if the apparent inconsistency could be mediated via an effect on anxiety. In theory, calmness would give rise to a decrease in blood pressure, whereas decreased restlessness in the long run may lead to an increase in blood pressure. Lower physical activity was observed in the fish oil-supplemented group at the 7-year follow-up and also at higher whole blood n-3LCPUFA-levels in the cross-sectional study of school children. Furthermore, the decrease in blood pressure after three months of n-3LCPUFA-supplementation in late infancy was found to correlate with an increase in number of episodes with quiet inattention in a free play test. We suggest that quite inattention may be interpreted as a measure of patience.

N-3 Fatty Acids and Arrhythmias

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In this presentation, we will review the evidence for prevention of sudden cardiac death and atrial fibrillation with n-3 polyunsaturated fatty acids. The primary focus will be on the effects of EPA and DHA. Secondarily, we will explore available evidence on alpha-linolenic acid (ALA).

Animal experimental and early clinical evidence provided a compelling hypothesis that DHA and EPA have anti-arrhythmic effects. This has led to several randomized clinical trials examining the effects of EPA and DHA supplements on arrhythmic outcomes, including: (1) recurrent ventricular tachycardia/ventricular fibrillation in patients with implantable cardio defibrillators; (2) total and sudden cardiac death in high risk patients (patients with a prior myocardial infarction, established congestive heart failure or angina); (3) incident atrial fibrillation following cardiac surgery; and (4) recurrent atrial fibrillation post-cardioversion in patients with persistent atrial fibrillation. Patient population, dose of DHA and EPA, concurrent drug therapies, comparison group, background diet, and expected effect size and power are some of the issues to consider in exploring the conflicting clinical trial results.

There are no randomized clinical trials examining the effects of DHA and EPA on arrhythmic outcomes among those without prior heart disease, and limited data on ALA and arrhythmic outcomes. To complement the clinical trial data, we turn to population-based epidemiological studies of DHA, EPA and ALA, assessed from diet questionnaires or from biomarkers. Totality of evidence will be summarized and gaps in our knowledge identified for future studies.

Effects of diet-induced decreases in brain DHA content on outcomes in a rat model of juvenile traumatic brain injury

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Children under 5 years of age are at particular risk of suffering a traumatic brain injury (TBI) and tend to have poorer outcomes than adults. Long-chain n-3 polyunsaturated fatty acids have been shown to have beneficial effects in a variety of models of neural injury in adult animals. This study examined whether diet-induced decreases in brain docosahexaenoic acid (DHA) affect sensorimotor outcomes in a rat model of TBI in toddlers with respect to severity and persistence of effects. Long-Evans rats (n=11-12/group) were raised from conception on a control diet (AIN-93G, α-linolenic acid-5.09 g/kg), or an n-3-deficient diet (α-linolenic acid-0.32 mg/kg). The n-3-deficient diet resulted in decreases in brain phospholipid DHA content of 25% and 54%, respectively, in the offspring from the 1st and 2nd matings of an individual dam. On postnatal day 17, rats received a unilateral controlled cortical impact injury to the sensorimotor cortex or sham surgery. Sensorimotor function was evaluated 1, 7, 14, 21, and 28 post-injury. TBI caused persistent deficits in forelimb preference indicated by increased forelimb laterality (P<0.05), and acute deficits in locomotor function indicated by decreased bouts of low mobility and low mobility distance on day 1 after injury (P<0.05). The n-3-deficient diet exacerbated these sensorimotor deficits after TBI in rats with a decrease in brain DHA of 54% (P<0.05), but not 25%. These findings indicate that a diet-induced decrease in brain DHA content contributes to poorer sensorimotor outcomes after TBI in juvenile rats. Furthermore, brain DHA level, rather than dietary n-3 PUFA content, appears to be the primary factor influencing TBI outcomes. This suggests that outcomes after TBI in young children

may be improved by ensuring adequate brain DHA levels through appropriate nutrition. Supported by NIH HD059939, HD02528, RR016475, and ES007079.

Pro-resolving mediators, resolvins and protectins in airway inflammation
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Acute inflammation in the lung is fundamentally important to host defense, but chronic or excessive inflammation leads to several important respiratory diseases. The resolution of inflammation is an active process that is directed, in part, by specialized pro-resolving mediators that are enzymatically derived from polyunsaturated fatty acids. In health, cell-cell interactions at the onset of acute inflammation establish biosynthetic circuits for specific chemical mediators, including resolvins and protectins, that later serve as agonists to orchestrate a return of the inflamed tissue to homeostasis. Understanding the cellular and molecular mechanisms for pro-resolving mediators in catabasis is providing new insights into tissue responses for resolution of airway inflammation in health and the pathophysiology of lung disease; as well as opportunities for therapeutic intervention.

E-series and D-series resolvins are enzymatically derived from the essential omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid, respectively. Protectin D1 is also derived from DHA. Resolvin E1 (RvE1), resolvin D1 (RvD1) and protectin D1 (PD1) are generated in murine lung and PD1 is present in human exhaled breath condensates. Receptors for RvE1 and RvD1, namely CMKLR1 and ALX/FPR2, are expressed in murine lung and are dynamically regulated with airway inflammation. Evidence will be presented for these representative members of a growing family of specialized pro-resolving mediators to demonstrate their protective actions in the regulation of airway inflammation during innate and adaptive immune responses to mucosal injury, infection and allergen.

Dietary Gangliosides decreased HBD-2 and IL-23 level in a Caco-2 cell model of IBD by improving barrier function Independent of NF-kB and sPLA2 Activation
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Gangliosides are negatively charged glycosphingolipids that consist of a hydrophobic ceramide and a hydrophilic oligosaccharide chain bearing one or more sialic acid. Gangliosides are found in human milk and cow's milk. Animal studies indicate that gangliosides modulate the immune system in weanling mice and may protect against infection. Both animal and human cell studies suggest that gangliosides have anti-inflammatory effects by modulating cytokine expression, such as decreasing tumor necrosis factor- α (TNF-α) , prostaglandin E2 (PGE2) and leukotriene B4 (LTB4). The objective of this study was to use a human epithelial cell model (Caco-2 cells induced by LPS) to investigate benefits of ganglioside supplementation on IBD signaling mechanisms. Gangliosides were extracted from milk powder and used as the ganglioside supplement in cell cultures. Epithelial barrier function was examined by measuring Trans Epithelial Electric Resistance. HBD-2, IL-23, NF-kB and sPLA2 (secretory phospholipase A2) was determined by ELISA methods. Results indicated that supplementing ganglioside to the cells in culture significantly decreased inflammatory HBD-2 and IL-23 level by 24% and 25%, respectively compared with the LPS treatment, and improved the defective epithelial barrier function. However, sPLA2 level in the apical and basolaterial medium was significantly increased both by ganglisode supplement and LPS treatment. In conclusion, the present study indicates that ganglioside supplementation in cell culture may have promising potential beneficial effects on IBD by decreasing inflammatory signaling and improving epithelial barrier function. The anti-inflammatory effect observed is independent of sPLA 2 and NF-kB activation.

Modulation of plasma fatty acid ethanolamide levels and physiological parameters as a function of dietary fatty acid composition in healthy humans

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Fatty acid ethanolamides (FAE) exist as regulators of energy and satiety, however, the impact of dietary FA composition on FAE in humans remains unknown. The objective was to examine the effects of diets enriched in flaxseed oil (FXCO) or high-oleic canola oil (HOCO) versus a Western fat (WF) blend on palmitoylethanolamide (PEA), oleoylethanolamide (OEA), linoleoylethanolamide (LEA), alpha-linolenoylethanolamide (ALEA), arachidonoylethanolamide (AEA), and docosahexaenoylethanolamide (DHEA) concentrations in plasma. Using a randomized crossover design, 36 hyperlipidemic subjects consumed 3 isoenergetic diets for 29d enriched in FXCO (20.6g/dALA), HOCO (2.4g/d ALA), or WF (1.3g/d ALA). On days 28 and 29, fasting blood was collected and stored. Body composition was assessed using DEXA and energy expenditure (EE)/substrate oxidation measured by indirect calorimetry. FAE were determined using UPLC-MS/MS with appropriate deuterated-FAE standards. Plasma OEA levels were highest ($p<0.001$) after HOCO (2.78 ± 0.15 ng/ml (SEM) feeding compared with FXCO (2.14 ± 0.15 ng/ml) and WF (2.19 ± 0.15 ng/ml). Plasma ALEA levels were highest ($p<0.001$) after FXCO (0.11 ± 0.01ng/ml) compared with HOCO (0.03 ± 0.01ng/ml) and WF (0.02 ± 0.01ng/ml). No actions of diet on any other FAE were observed. Within each diet, plasma FAE levels failed to correlate with corresponding FA. However, with FXCO feeding, DHEA levels correlated with BMI ($r=0.37$, $p<0.05$), resting EE ($r=0.42$, $p<0.02$) and post-breakfast carbohydrate ($r=0.34$, $p=0.05$) and fat ($r=-0.39$, $p=0.05$) oxidation rates. With HOCO feeding, AEA levels correlated with total fat mass ($r=-0.39$, $p<0.05$), gynoid fat ($r=0.37$, $p<0.05$), carbohydrate ($r=-0.47$, $p<0.01$) oxidation rate, and total EE ($r=0.38$, $p<0.05$). Results demonstrate that FAE reflect dietary, but not plasma, FA composition. However, FAE do associate with energy metabolism in humans, underscoring the role of these metabolic regulators in energy pathways. (Support by Canola Council of Canada)

Insights into the functionality of alpha linolenic acid: lessons from model systems

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The independent and direct effects of alpha linolenic acid (ALA) on health and disease remain equivocal. While there is evidence that ALA may have beneficial effects for various chronic diseases, it is not possible to discern whether effects are also due to conversion of ALA to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The recent development of the delta 6 desaturase knock out (D6KO) mouse model has potential to shed light on the biological role of ALA. The delta 6 desaturase enzyme is the rate limiting step in the metabolism of ALA to EPA and DHA. Thus, the loss of this enzyme enables the isolation of ALA effects independent of its conversion. Growing evidence suggests that essential fatty acids play a role in the development of non-alcoholic fatty liver disease, which is a growing health concern. The objective of the study was to determine if α-linolenic acid (ALA) can independently prevent hepatic steatosis and inflammation using the D6KO mouse. Experimental groups included male wild type (WT) or D6KO mice fed a high fat diet (30% of energy) containing either: lard (LD), canola oil (CD,11% ALA), flax oil (FD,50% ALA), or fish oil (MD, n-3 HUFA) (n=4-7/group) for 8 or 20 weeks. At 8 weeks mean hepatic inflammation scores for CD and FD groups (both WT and KO) were intermediate between LD and MD. Within genotypes, D6KO FD and CD groups had higher hepatic steatosis scores relative to WT mice. But, FD and MD D6KO groups had lower liver lipid mass relative to LD-fed D6KO mice. Fattier livers in D6KO mice were associated with decreased adiposity at 8 and 20 weeks. Gas chromatography confirmed the enrichment of ALA and the lack of n-3 HUFA in D6KO mice. The similar effects observed between WT and D6KO mice suggest that ALA has direct effects on inflammation independent of conversion; however, the lack of conversion to HUFA resulted in the development of fatty liver. (Supported by Canola Council of Canada, NSERC, CFI/ORF to D.Ma and OGS to J.Monteiro)

Hypertriglyceridemia and cardiovascular disease risk: Biology to bedside

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Elevated fasting and postprandial triglyceride (TG) levels are associated with greater risk for atherosclerotic cardiovascular disease (CVD). Possible pathophysiologic links for this association include atherogenicity of TG-rich remnant lipoprotein particles, especially in the postprandial state, increased levels of small, dense low-density lipoprotein particles in hypertriglyceridemic patients, and the association of hypertriglyceridemia with other metabolic disturbances, including insulin resistance, inflammation and hypercoagulation. In recent years the prevalence of hypertriglyceridemia has increased markedly in North America, in parallel with the rising incidence of obesity. Despite several decades of research on lipid-altering drug therapies, no large-scale, prospective outcomes trial has been completed to evaluate the efficacy of lipid-altering drug therapy in patients specifically selected for the presence of hypertriglyceridemia. The best available evidence for therapy in hypertriglyceridemic patients is from subgroup analyses of clinical outcomes trials with statins, fibrates, and omega-3 fatty acids. These results suggest that individuals with elevated TG, along with below-average levels of high-density lipoprotein cholesterol, benefit from lipid modifying therapies. Additional clinical events data are needed to provide a more evidence-based rationale for clinical lipid management in hypertriglyceridemic patients, and to further explore the importance of fasting vs. nonfasting TG measurements in the prediction of CVD risk.

DHA supplementation of preterm infants: the relevance of dose and timing

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Preterm infants are born before they have had the opportunity to accumulate a full complement of docosahexaenoic acid (DHA, 22:6n-3) and are at greater risk of DHA depletion than their term-born counterparts. Although the primary focus regarding the early dietary supply of DHA has focussed on neurodevelopmental outcomes, other physiological and organ functions may be affected by DHA supply. The DINO trial investigated the effect of increasing for dose of dietary DHA from approximately 0.3% of total fatty acids to approximately 1% of total fatty acids during the neonatal period on neurodevelopmental and clinical outcomes of preterm infants born < 33 weeks' gestation. The aim of DHA supplementation was to achieve the level of DHA accumulation that would occur in the womb. We showed that increasing the dietary DHA concentration during the neonatal period resulted in fewer infants with mild and significant cognitive delays at 18 months of age, although there were no differences in the mean developmental quotient scores. Significant treatment by sex and treatment by birth weight interactions were noted indicating that boys and girls respond differently to DHA supplementation and that birth weight may also be important in predicating the DHA responsiveness. In secondary analyses, high DHA supplementation also reduced the incidence of bronchopulmonary dysplasia in boys and in infants from the randomisation strata born weighing <1250g. Further work is needed to better define the sub-groups of children who will benefit from DHA supplementation during the perinatal period.

Investigating the role of bioactive lipids in inflammatory bowel disease

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Introduction: Ulcerative colitis (UC) is a relapsing remitting disorder of the colon. Disease pathology and clinical course are variable but associated with significant physical morbidity and socioeconomic burden. Although the aetiology of UC is unknown, current pathological evidence suggest an important role for proinflammatory cytokines and lipid mediators in the initiation and perpetuation of UC. Overexpression of cyclo-oxygenase (COX) and lipoxygenase (LOX) enzymes, which synthesize bioactive lipids from fatty acids have been reported in mucosal biopsies of UC patients. However, a detailed characterisation of intracellular and extracellular pools of lipid mediators and their role in UC is currently lacking.

Procedure: Mucosal biopsies were taken from active UC patients (n=66) within endoscopically inflamed and normal mucosa, and age-sex matched external controls. Disease activity, endoscopic appearance and histopathology were graded. Lipidomics analysis was performed to profile bioactive lipids and measure fatty acid bioavailability in the mucosal biopsies using an automated data-dependent mass spectrometry assay. For statistical analysis, we used Wilcoxon's Signed Rank Test at a given significance level of 0.001 and confidence interval of 0.975.

Results and conclusions: There was no significant difference between endoscopically normal mucosa from UC patients and external controls. The pro-inflammatory COX-related metabolite, prostaglandin E2, was upregulated in endoscopically inflamed tissue. The neutrophil chemoattractant LTB4 was not detectable; however, the level of 5- hydroxy-eicosatetraenoic acid (5-HETE) was elevated significantly in the inflamed tissue. Other LOX-related lipid mediator,15- hydroxy-eicosatetraenoic acid (15-HETE) was increased significantly in the inflamed mucosa, suggesting a "cross-talk" between the COX and LOX pathways within arachidonic acid cascades. This was in agreement with significant elevation in bioavailability of arachidonic acid. Using comprehensive lipidomics approach, we have identified several potential lipid mediators associated with colitis pathology. The resulting discoveries could further advance our understanding of UC, potentially leading to improved disease classification to facilitate future therapeutic approaches.

Fish oil improves body composition and response to chemotherapy in patients with lung cancer

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Background and Objectives: Patients with lung cancer are at high risk for malnutrition and even mild weight loss has been associated with decreased median survival and poorer response to chemotherapy. The purpose of this research was to determine if supplementation with fish oil reduces weight and muscle loss and enhances the effectiveness of chemotherapy in lung cancer.

Methods: Patients with non-small cell lung cancer who were newly diagnosed were accrued to the either receive standard of care (SOC, no intervention) or to receive fish oil (FO) supplementation during chemotherapy until the end of treatment.   Blood was collected at baseline and throughout chemotherapy treatment. Toxicity from the chemotherapy and response to chemotherapy were also determined. Plasma fatty acids were isolated and quantified using gas liquid chromatography. Body composition was assessed using diagnostic computed tomography (CT) images when available.

Results: The majority of patients were over 60 years old, had advanced disease and heavier body weights. In the SOC group, depletion of n-3 fatty acids was prevalent and associated with low muscle mass, accelerated loss of skeletal muscle and adipose tissue. Supplementation with fish oil provided a benefit over SOC on weight, and skeletal muscle; 69% of patients in the fish oil group maintained or gained muscle and weight compared to 29% of patients in the SOC group. Supplementation with fish oil also resulted in a 2-fold improvement in chemotherapy efficacy compared to SOC: 60% of patients had a reduction in tumor size and there was a trend towards greater 1-year survival in the fish oil group.

Conclusion: These results demonstrate the potential of fish oil to improve the care and treatment of patients with lung cancer.

Improved outcome after spinal cord injury in transgenic mice with high levels of endogenous omega-3 polyunsaturated fatty acids

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Omega-3 polyunsaturated fatty acids (PUFAs) have been shown to have therapeutic potential in a variety of neurological disorders, including acute traumatic injury of the spinal cord. We investigated whether the

neuroprotective effect of these compounds after spinal cord injury (SCI) could also be seen when their level is raised in tissues prophylactically, prior to SCI. In this study we used transgenic fat-1 mice to examine whether enriching the spinal cord tissue in endogenous omega-3 PUFAs prior to trauma, has an effect on the outcome after compression SCI. The results demonstrated that after thoracic compression SCI, fat-1 mice display better functional locomotor recovery compared with the wild-type (WT) mice on a high omega-6 diet (high omega-6 PUFAs in tissues), and WT mice on a normal diet (controls). This improved neurological outcome is associated with a significant increase in neuronal and oligodendrocyte survival and a decrease in axonal non-phosphorylated neurofilament loss. The protection from SCI in fat-1 mice was also correlated with a reduction in neuroinflammation, i.e. a clear decrease in microglia/macrophage activation and in pro-inflammatory mediators. In vitro experiments in dorsal root ganglia primary sensory neurones further demonstrated that a fat-1 tissue background confers robust neuroprotection against a combined mechanical stretch and hypoxic injury. In conclusion, both the in vivo and in vitro results in this study support the hypothesis that a raised omega-3 PUFA level and an altered tissue omega-6/omega-3 ratio prior to cord neurotrauma leads to a much improved neurological outcome after SCI and to significant tissue protection.

Ganglioside increases infectivity of PC-3 prostate cancer cells to adenovirus in vitro

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Prostate cancer is the 2nd most common cancer in North American men. Advanced disease has a poor prognosis and requires prompt intervention. Therefore, research into novel treatment for aggressive cancer is of particular interest. Adenovirus-mediated gene therapy is a potential therapeutic agent for treatment of prostate cancer. Several studies have shown that lipids present in diet can affect the safety and efficacy of cancer treatments. Gangliosides are glycosphingolipids that regulate many processes including cell death, protein localization and endocytosis. This study assessed the role of a milk fat fraction enriched with ganglioside in sensitizing prostate cancer cells to adenovirus-mediated gene therapy in vitro. Healthy (RWPE-1) and malignant (PC-3) prostate cells were cultured with or without 10 µg/mL mixed ganglioside treatment for 48 hours. Cell cultures were then overlaid with 0, 10, 20, 50, or 100 plaque forming units (pfu)/cell of GFP-expressing adenovirus under the control of cytomegalovirus promoter (AdBM116GFP) for 24 hours. At 100 pfu/cell, GFP detection in RWPE-1 cells treated with ganglioside was 20% ($p<0.01$) lower than in untreated RWPE-1 cells. Ganglioside treatment resulted in increased adenoviral GFP expression in PC-3 cells compared to cells incubated without supplemental ganglioside. GFP detection was 27% ($p<0.02$), 47% ($p<0.01$), 24% ($p>0.02$), and 9% ($p<0.22$) higher at 10, 20, 50, and 100 pfu/cell respectively in ganglioside-treated PC-3 cells than in untreated PC-3 cells. These effects appear to be mediated by the influence of ganglioside on the primary adenovirus receptor: coxsackie and adenovirus receptor. Ganglioside functions as an effective adjunct to adenovirus-mediated gene therapy as ganglioside pre-treatment increased infectivity of adenovirus in malignant PC-3 cells, but not in RWPE-1 cells.

Long chain omega-3 fatty acid supplementation improves cognition and mood in older Australians with memory problems

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Background - Suboptimal Omega-3 (n-3) polyunsaturated fatty acid (PUFA) status assessed through erythrocyte PUFA levels may contribute to both depression and dementia. Depressive symptoms may contribute to an increased rate of progression to more severe forms of dementia. An increase in n-3 PUFA status through consumption of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may alleviate some of the cognitive and depressive symptoms associated with mild cognitive impairment (MCI).

Objective – To compare effects of supplementation with DHA-rich and EPA-rich oils versus safflower oil on cognition, memory, mood and overall quality of life in older adults with MCI.

Design - Fifty adults ≥ 65 years with MCI were recruited for a 6-month double-blind placebo-controlled parallel trial. Volunteers were randomly allocated to consume an EPA-rich oil (1670 mg EPA + 160 mg DHA/day), DHA-rich oil (400 mg EPA + 1550 mg DHA/day) or linoleic acid (safflower oil, 2200mg LA/day). Erythrocyte PUFA status, assessments of memory, cognition, self-rated quality of life, health and mood using the geriatric depression scale (GDS), were measured at 0 and 6 months.

Outcomes – 38 volunteers completed the trial. After 6 months, DHA supplementation improved verbal fluency (initial letter fluency) ($p < .05$), and both EPA and DHA supplementation improved depression scores (GDS) compared with LA supplementation (EPA: $p < .05$, DHA: $p < .01$). Improvements in depressive symptoms were associated with increased erythrocyte DHA+EPA ($r = .39$, $p < .05$) over 6 months. Improved self-reported physical health was associated with increased erythrocyte DHA ($r = .39$, $p < .05$).

Conclusion - Increasing n-3 PUFA status by increased intake of EPA and DHA may improve mood and cognition in older adults with MCI. The effect of these improvements on dementia risk, cognitive decline rate and quality of life in older people needs to be investigated further.

Greater sensitivity of plasma lipids and CRP to dietary fat manipulation in APOE4 carriers: insights from the SATgenε study

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The aetiology of the highly heterogeneous response of plasma lipids to dietary fat manipulation is relatively unknown. The aim was to determine the effects of diets differing in fat quantity and composition on lipid-related cardiovascular disease biomarkers according to apolipoprotein (APO)E genotype. Participants (mean (SD) age 51 (9) years and BMI 26.0 (3.8) kg/m2) prospectively recruited according to APOE genotype (n=44 E3/E3, n=44 E3/E4), followed a sequential dietary intervention in which they were assigned to a low fat (LF, 24% energy (E) from fat, 8%E saturated fat (SFA)), high fat-high saturated fat (HSF, 38%E fat, 18%E SFA), and HSF with 3 g/d docosahexaenoic (HSF-DHA) diets, each for a 8 wk period, in the same order. Fasting blood samples were collected at the end of each of the intervention diets. A significant diet*genotype interaction was observed for plasma triglycerides (TG). In both genotype groups, TG was significantly lower following HSF-DHA relative to the LF (17% E3/E3, 30% E3/E4) and HSF (20% E3/E3, 24% E3/E4) diets, with an 8% lower TG also found after the HSF versus LF in APOE4 carriers. Although no significant diet*genotype interaction was observed for low-density lipoprotein cholesterol (LDL-C), subgroup analysis based on median baseline TG values, revealed higher LDL-C following the HSF (3.62 mmol/l) and HSF-DHA (3.58 mmol/l) diets than the LF diet (3.27 mmol/l), in the APOE3/E4 individuals with a TG < the median (1.1 mmol/l). Furthermore, a significant APOE genotype*diet interaction was evident for CRP with diet composition only influencing CRP concentrations in APOE4 carriers. In conclusion, our results suggest a greater sensitivity of plasma lipids and CRP to dietary fat quantity and composition in APOE4 carriers. Although both genotypes benefited from the TG lowering effects of DHA, E3/E4 individuals showed a more marked response, indicating additional hypotriglyceridaemic benefit in this large population subgroup.

Anomalous Permeation Through Membranes with Coexisting Liquid Ordered/Liquid Disordered Phases

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A commonly proposed physical mechanism for lateral domain segregation in biological membranes is the cholesterol-induced liquid ordered (lo) phase and coexistence of lo and liquid disordered (ld) phases. With the goal of understanding some of the properties of these phases we examined the permeation of water and protons across membranes consisting of pure lo, pure ld and mixed lo/ld phases. Compositions were selected based on ternary phase diagrams for POPC- sphingomyelin (SM)-cholesterol and DOPC-DSPC-cholesterol. LUVs loaded with carboxyfluorescein (cf) were formed via extrusion at 70 0C. Stopped-flow fluorescence spectroscopy was used to measure changes in cf fluorescence induced by rapidly applied osmotic or pH gradients. In both ternary systems water permeation was highest for pure ld membranes and a factor of ~15 lower in the pure lo phase. Values for

membranes with coexisting lo/ld phases were only about 2-fold lower than the ld phase. An interesting exception was in POPC-SM-chol in the lo/ld coexistence region where the connected phase is lo, where water permeation was similar to that observed in pure lo. Proton permeation was lowest in both pure ld phases. In the lo/ld coexistence region proton permeation increased ~20 fold, but in pure lo membranes it was only ~2.5 times higher than in pure ld. Increases in proton permeation with increasing cholesterol or SM have been reported previously. These results strongly suggest that this increased permeability is due to lo/ld coexistence. We also used time-resolved measurements of DPH fluorescence to characterize acyl chain packing and relative headgroup packing in all membranes. These results did not suggest variation in acyl chain packing as an underlying cause of the anomalously high proton permeation in lo/ld membranes. Comparison of the two pure lo phases and the two pure ld phases show that these two ternary systems produce somewhat different acyl chain packing.atment

Novel mass spectrometry techniques for the full structural characterisation of molecular lipids

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The recent expansion in the field of lipidomics has been driven by the development of new mass spectrometric tools and protocols for the identification and quantification of molecular lipids in complex matrices. Nevertheless, standard mass spectrometry techniques such as collision-induced dissociation (CID) are limited in their ability to identify several important structural isomers, i.e. double bond position, double bond stereo chemistry (cis vs. trans), chain branching and the site of fatty acid esterification to the glycerol backbone (sn position). We have developed several novel techniques for lipid structural analysis termed ozone-induced dissociation (OzID) and radical directed dissociation (RDD) that are helping to resolve the limitations.

In OzID analysis, lipid ions are mass-selected in an ion-trap mass spectrometer and allowed to react with ozone vapour with the resulting chemical induced fragment ions allowing localization of double bonds. Recent implementation of this technique on a tandem linear ion-trap mass spectrometer (QTRAP) has significantly decreased the acquisition time for OzID analysis, allowing data acquisition on a timescale compatible with liquid chromatography. Through the combination of CID and OzID we are also able to glean information regarding sn position.

RDD was originally developed by Ly and Julian for the characterization of peptides [1]. We have recently shown that electrospray ionization of lipids in the presence of 4-iodoaniline or 4-iodobenzoic acid can produce adduct ions in positive or negative ion mode, respectively. Once formed, these adduct ions can be mass-selected and subjected to either CID where they give rise to predictable product ions or photodissociation giving rise to a radical ion by cleavage of the carbon-iodine bond. Subsequent CID of the nascent radical ion gives rise to a rich fragmentation chemistry that capable of identifying double bond position and sites of chain branching.

1. Ly, T. and R.R. Julian, J AmChem Soc, 2007. 130: 351-358.

FADS gene variants are associated with plasma total and HDL cholesterol in 2-year-old children from the KOALA Birth Cohort Study.

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Background and aims. Genetic variants (SNPs) in the genes coding for fatty acid desaturases 5 and 6 (FADS1 FADS2 gene cluster) have been associated with blood lipids levels in adolescents and adults. To the best of our knowledge, no study has yet reported whether these associations are already present in children. Therefore we aimed to investigate whether FADS SNPs were associated with blood lipids in 2-year-old infants.

Material and methods. The study included 539 children from the KOALA Birth Cohort Study (www.koala-study.nl) with buccal swabs for DNA isolation available and blood collected at 2 years of age. Five FADS SNPs were genotyped: rs174545, rs174546, rs174556, rs174561, rs3834458. Total plasma cholesterol and HDL cholesterol were analyzed with enzymatic kits. Statistical analyses were done using multiple linear regression, correcting for children's sex.

Results. Children homozygous for the rs174545 major allele had a mean total cholesterol concentration of 3.90 mmol/L, whereas children homozygous for the minor allele had a concentration 7% lower (3.63 mmol/L; p=0.002). Heterozygous children had concentrations in between (3.75 mmol/L, p=0.011). Associations found for the other four SNPs were slightly weaker but still statistically significant. Similarly, HDL cholesterol was lower in homozygous for the minor allele vs. major allele carriers, but the associations were not as strong as with total cholesterol and reached statistical significance only in homozygous minor vs. homozygous major allele of rs174545, rs174546, and rs3834458. Correction for sex did not essentially alter the results

Conclusions. FADS SNPs are associated with total cholesterol and, less strongly, with HDL cholesterol in 2-year-old children. We might speculate that FADS gene variants might contribute to tracking of lipid levels over life. C Moltó-Puigmartí Supported by a fellowship from Fundación Alfonso Martín Escudero

N-3 polyunsaturated fatty acids antagonize Th17 cell biology during experimental colitis

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During inflammatory bowel disease (IBD), Th17 cells are implicated in disease initiation and progression, however, effective therapies to maintain IBD remission remain undetermined. Although the mechanisms are not fully elucidated, ~50% of IBD subjects utilize oral complementary/alternative medicines, e.g., fish oil (FO), containing n-3 polyunsaturated fatty acids (PUFA). Utilizing both dietary (4% FO) and genetic models (Fat-1 transgenic mouse that synthesizes n-3 PUFA de novo), we show that n-3 PUFA directly antagonize multiple facets of Th17 cell biology in diverse complementary mucosal inflammation model systems. Specifically, in unchallenged C57BL/6 mice fed a 4% FO diet, splenic CD4+ T cells exhibited a reduced capacity to polarize into a Th17 cell phenotype (CD4+ IL-17A+, P=0.046) versus T cells from mice fed an n-6 PUFA enriched diet. In addition, Fat-1 mice exhibited (i) reduced colitis (inflammation scores) in response to repeated cycles of dextran sodium sulphate (DSS), and (ii) a reduced % of colonic mucosal (local) and splenic (systemic) Th17 cells (P=0.03) compared to wild-type mice. Further, the reduced percentage of systemic Th17 cells in Fat-1 mice was confirmed in the T cell driven trinitrobenzenesulfonic acid (TNBS)-colitis model (P=0.03). Fat-1 mucosal mRNA expression of IL-17F and IL-21 was suppressed (P<0.05), indicative of reduced Th17 cell function and differentiation/polarization, respectively. Additionally, mRNA expression of the Th17 cell suppressive cytokine, IL-27, was upregulated in Fat-1 mice (P=0.04). Similarly, in a chronic colitis (DSS)/carcinogenesis (azoxymethane) model, dietary n-3 PUFA altered the colonic cytokine mRNA profile in a manner consistent with reduced Th17 cell function and polarization by decreasing expression of IL-17, IL-23 and IL-6 (P<0.05). Collectively, these results demonstrate that n-3 PUFA antagonize multiple aspects of Th17 cell biology in diverse colonic mucosal inflammation models, thereby emphasizing a potent and reproducible emerging mechanism to reduce mucosal inflammation.

Omega-3 Fatty Acid Supplementation Increases Plasma Resolvins and Protectins in Patients with Chronic Kidney Disease

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Background: Patients with chronic kidney disease are at increased risk of cardiovascular disease (CVD). Modifiable risk factors that contribute to the increased risk include hypertension, endothelial dysfunction, dyslipidaemia, insulin resistance and a pro-inflammatory state. Omega-3 fatty acids protect against CVD via multiple mechanisms including suppression of inflammation. Resolvins and protectins derived from

eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have potent anti-inflammatory actions and have been implicated as mediators of resolution of inflammation.

Aim: To determine whether omega-3 fatty acid supplementation increases plasma levels of resolvins and protectins in patients with chronic kidney disease stages 3-4.

Methods: 85 patients were randomised to supplement their normal diet with either omega-3 fatty acids (4g/day, providing 1840mg EPA and 1520mg DHA) or control (4g/day olive oil). Fasting plasma samples were collected at baseline and after 8 weeks. Resolvins and protectins were purified by solid phase extraction with LTB4-d4 as internal standard and analysed using LC-MS-MS.

Results: Patients were aged 56.5 yrs; BMI 27.3 kg/m2; supine blood pressure 125.0/72.3 mmHg; and GFR 35.8 ml/min/1.73m2. Seventy four patients completed the intervention. Omega-3 fatty acids significantly reduced triglycerides, 24 hr blood pressure and heart rate. At baseline there were no correlations between patient indices of renal function or biochemistry and resolvins and protectins. Following supplementation several resolvins were significantly increased: (±)18-HEPE (85±40pg/ml to 422±39pg/ml, P<0.001) from EPA, and 17(S)-HDHA (183±26pg/ml to 335±25pg/ml, P<0.001) and 17(S)–RvD2 (26±2pg/ml to 33±2pg/ml, P=0.036) from DHA. 17(R)–RvD1; PD1 and 10(S),17(S)-dHDHA were not altered.

Conclusions: The increase in resolvins following omega-3 fatty acid supplementation would likely significantly attenuate the pro-inflammatory state in these patients. These findings together with improvements in other CVD risk factors, lends support for increased omega-3 fatty acid intake in patients with chronic kidney disease.

Fatty Acid Desaturase Activity and Prostaglandin E2 Production in Colorectal Cancer

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Colorectal cancers (CRC) overproduce the arachidonic acid-derived eicosanoid prostaglandin E2 (PGE2), which promotes tumor proliferation and invasiveness. Δ6 desaturase (D6D) and Δ5 desaturase (D5D) convert dietary linoleic acid into arachidonic acid. It is not known if desaturase activity might impact colorectal cancer risk or production of PGE2. We measured erythrocyte phospholipid polyunsaturated fatty acid (PUFA) percentages and urinary PGE2 metabolite (PGEM) in 135 CRC cases and 134 controls in a nested-case control study within the Shanghai Women's Health Study. We calculated D6D and D5D activity indirectly by using fatty acid product-to-precursor ratios. We calculated Spearman partial correlation coefficients for D6D and D5D activity and urinary PGEM levels adjusting for age, body mass index, aspirin use, and erythrocyte phospholipid percentage of long chain (≥ 20 carbons) n-3 PUFAs. Conditional logistic regression models were constructed to determine the association between calculated D6D and D5D activity, categorized into quartiles, and CRC risk. Logistic regression models were adjusted for the same confounders as above along with smoking status and education level. In CRC cases, urinary PGEM levels were positively correlated to D6D activity (r = 0.28, P-value = 0.002) and negatively correlated with D5D activity (r = - 0.23, P-value = 0.01). In controls there was no correlation found between urinary PGEM levels and D6D (r = -0.04, P-value = 0.67) or D5D activity (r = 0.06, P-value = 0.52). Increasing D6D activity was associated with an increased risk of CRC (OR 2.47 [95% CI 1.01, 6.04]Q2 vs Q1, OR 1.68 [95% CI 0.61, 4.69]Q3 vs Q1, OR 1.94 [95% CI 0.69, 5.45]Q4 vs Q1). No statistically significant associations were found between D5D activity and CRC risk. Colorectal cancer risk may be influenced by fatty acid desaturase activity and this effect may be mediated through PGE2 production.

New insights into essential function of arachidonic acid (ARA) revealed with delta-6 desaturase (D6D)-null mice

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In mammals, ARA plays important physiological roles as precursors of eicosanoids and endocannabinoids. However, a full scope of ARA function is yet to be elucidated. A major obstacle of elucidating ARA function is endogenous synthesis of ARA from linoleic acid (LA). It is not possible to create ARA deficiency by dietary manipulation without depleting LA, which is an essential component of skin ceramides. To overcome this obstacle, we created mice lacking the D6D gene that encodes the first step of ARA synthesis. When D6D-/- mice were fed a diet lacking ARA and docosahexaenoic acid (DHA) but containing sufficient LA and alpha-linolenic acid (ALA), they exhibited fatty liver, intestinal ulcer, severe dermatitis, altered macrophage function and male infertility. In liver, lipid droplets were primarily localized in a periportal area. Intestinal ulcer was observed in duodenum and the ileo-cecal junction, being more prominent in the latter. The dermatitis of the D6D-/- was ulcerative and distinct from dry, scaly dermatitis observed in classic essential fatty acid deficiency. Peritoneal macrophages from D6D-/- showed elevated cholesterol biosynthesis and reduced paraoxonase 2 expression. D6D-/- males exhibited impairment of sperm formation. Dietary ARA was required to reverse these phenotypes except for the male fertility, which was fully rescued by dietary DHA, whereas ARA was only partially effective. Although some of these phenotypes are likely due to reduced eicosanoid formation, the exact mechanism is yet to be elucidated. In conclusion, previously unknown ARA functions were identified using theD6D-null mouse, which will continue to be a useful tool to investigate the mechanism underlying ARA functions.

Opposite effects of dietary n-6 and n-3 PUFA during metabolic syndrome on liver lipid biosynthesis and insulin sensitivity

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Metabolic syndrome (MS) characterized by insulin resistance, obesity and dyslipidemia is accompanied by severe lipid metabolism perturbations and chronic low-grade inflammation. However, many unresolved questions remained regarding the regulation of inflammatory response and specifically the different steps leading to synthesis and release of bioactive inflammatory lipid mediators such as eicosanoids. The mechanisms responsible for the onset of MS involve environmental factors and among them, dietary lipids play an important role, especially n-3 and n-6 PUFA, whose roles are still controversial.

Because the liver has a central role in glucose and lipid metabolisms, we investigated here hepatic modifications of PUFA biosynthesis and signalling during the establishment of MS. Three-month-old Zucker fatty rats were used as model of MS – in comparison with their lean littermates – and were fed either a n-3 (flaxseed oil) or a n-6 (sunflower oil) rich diets from gestation until sacrifice.

Our results showed strong perturbations of hepatic PUFA biosynthesis and signalling pathways (increase of desaturase and elongase expressions, decrease of cPLA2 expressions) in insulin resistant rats fed n-6 rich diet compared to lean animals. Moreover, the n-6 rich diet significantly increased the glucose intolerance and tended to potentiate the PUFA signalling and biosynthesis perturbations observed in animals fed a standard diet. On the contrary, rats fed the n-3 rich diet showed strong improvement of hepatic parameters and normalized insulin sensitivity.

In conclusion, this study evidences that n-3 and n-6 rich diets modulate differently lipid liver biosynthesis and insulin sensitivity in the early state of MS. It suggests new nutritional targets for the improvement of insulin parameters and pathways leading to eicosanoid precursors in such pathological conditions.

Docosahexaenoic acid algal oil reduces arthritis severity and inflammation more effectively than fish oil in preclinical models of arthritis

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The long-chain omega-3 polyunsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have potent anti-inflammatory activity. Although the efficacy of fish oil in arthritis has been examined, the anti-arthritic effect of DHA has yet to be investigated. The objective of these studies was to determine the anti-inflammatory activity of DHASCO oil, a DHA-rich algal oil that contains no EPA, in murine models of arthritis. In a collagen induced arthritis (CIA) model, a widely used model for human rheumatoid arthritis, treatment with DHASCO oil significantly reduced arthritis severity, delayed disease onset and decreased arthritis incidence whereas fish oil had no significant effect. There was an overall reduction in inflammation and bone and cartilage damage in the joints of animals treated with DHASCO oil. DHASCO oil treatment modulated the humoral and cellular immune response to collagen by decreasing both the production of pathogenic anti-collagen antibodies and collagen-specific proliferation by splenocytes ex vivo. Plasma arachidonic acid levels were reduced more significantly upon treatment with DHASCO oil than fish oil. These results demonstrate that DHASCO oil is more potent than fish oil in reducing arthritis and suggests that DHA may be a useful intervention strategy against signs of inflammatory arthritis.

Xanthophylls, N-3 fatty acids and retinal aging

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Both n-3 fatty acids and the macular pigment xanthophylls, lutein and zeaxanthin, have been linked to lower risk for age-related macular degeneration (AMD), the leading cause of vision loss in the elderly. We examined the effects of these nutrients on retinal structure and function in rhesus monkeys. These animals provide a uniquely valuable model AMD because they possess a macula and commonly develop age-related maculopathy that shares genetic risk factors with the human disease; however, they almost never spontaneously develop AMD's most advanced atrophic or neovascular stages. From birth until 14-16 years of age, 19 rhesus monkeys were fed semipurified diets lacking lutein and zeaxanthin, and therefore they had no macular pigment. Eight of these received a diet also deficient in n–3 fatty acids, and the remaining 11 received adequate n–3 fatty acids. Both groups developed drusen, the subretinal deposits that are the hallmark sign of AMD, several years earlier than monkeys on standard diets. Those deficient in n-3 fatty acids had an age-related reduction in rod sensitivity as measured by electroretinography (ERG). They also showed selective losses in the central retina of 1) the maximal rod ERG, indicating a loss of rod photoreceptors, and 2) the speed of dark adaptation. These functional changes parallel those seen in human aging and AMD. Some developed advanced atrophic AMD by 15-16 years of age (= 45-48 human years) that correlated with areas of increased lipofuscin. Given that atrophic macular disease is an extremely rare occurrence in monkeys, its appearance at a relatively young age in monkeys deficient in xanthophylls and n–3 fatty acids supports the role of these nutrients as significant factors for the prevention of macular disease.

Eicosanoids in skin inflammation: lessons from the sunburn response

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Skin displays an active metabolism of polyunsaturated fatty acids (PUFA) and this includes the formation of eicosanoids, octadecanoids and other bioactive lipids. Eicosanoids play vital roles in homeostatic mechanisms related to skin health and can mediate inflammatory events that develop in response to environmental factors or cutaneous disease. Ultraviolet radiation (UVR) in sunlight is a key environmental stressor impacting on skin health. Acute effects include sunburn, immune-suppression and development of photosensitivity, while repeated exposures lead to photoageing and photocarcinogenesis. Sunburn is an acute inflammatory response characterized clinically by erythema and histologically by dermal infiltrate of neutrophils. This self-limiting inflammation presents an accessible and convenient model applicable to

study cutaneous eicosanoids as well as other classes of lipid mediators. Mass spectrometry-based mediator lipidomics has allowed us to dissect the role of eicosanoids in sunburn inflammation. This approach revealed the involvement of a wide range of lipids that mediate the initiation and progress of the response. Furthermore, we have observed temporal changes and varying contributions of different eicosanoids occurring in a sequential manner, with COX-derived prostanoids intervening at the early stages of cutaneous inflammation and LOX-derived hydroxy fatty acids being more involved later on, with a possible contribution to the resolution phase. Interestingly, the skin of individuals who respond more readily to UVR and tend to burn and not tan, shows a late elevated production of PGE2 and 15-HETE when compared with individuals that are more resistant to sunburn, and following the same high UVR exposure. This finding suggests a possible role for 15-HETE and its metabolites in tempering skin inflammation. Finally, manipulation of skin fatty acids following nutritional supplementation with n-3PUFA has shown a reduction in the erythema response indicating that a systemic approach could convey protective activities through, at least in part, altering the ratio of n-3/n-6 PUFA-derived eicosanoids.

Stearoyl-CoA desaturase-1 Is Essential for Lipid Homeostasis in Skin

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Stearoyl-CoA desaturase (SCD) is a lipogenic enzyme that is expressed at high levels in several human tissues including skin and is required for the biosynthesis of oleate (C18:1n9) and palmitoleate (PA: C16:1n7) which are the major monounsaturated fatty acids of membrane phospholipids, triglycerides, wax esters and cholesterol esters. There are four SCD isoforms in mouse and two have been characterized in humans. We have generated mice with a skin-specific deletion of SCD1 (SKO) and found that the SKO mice develop sebaceous gland hypoplasia with progressive hair loss and decrease in sebaceous gland lipids as the mice age. Loss of SCD1 expression in the SKO mice is accompanied by a dramatic decrease in the levels of monounsaturated fatty acids and an increase in skin levels of retinol and retinoic acid as well as increased expression of retinoic acid-induced genes. Excess retinoid activity is implicated in the skin causes of alopecia and other skin diseases. Feeding diets containing high levels of oleate to the SKO mice does not increase the levels of OA in the skin and fails to restore the sebaceous lipids and normal hair growth found in the skin of the wild-type mouse. These observations demonstrate that endogenously synthesized monounsaturated fatty acids by SCD are important for retinol homeostasis and normal skin function. The research is relevant to a variety of human disorders that include acne, seborrheic dermatitis, and androgenetic alopecia.

Fatty acid metabolism in skin physiology; epidermal, sebaceous and subcutaneous involvements

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Skin lipids are of sebaceous and keratinocyte origin. The subcutaneous layer is mainly consisted of adipocytes. Human sebum is the predominant secreted mixture of lipids, mainly triglycerides, wax esters, squalene, and smaller amounts of free fatty acids, cholesterol and cholesterol esters. Elevated sebaceous lipid synthesis is a major factor involved in acne. The sebaceous gland synthesizes lipid species that are not found in other cell types and tissues of the body. Complexity and uniqueness characterizes sebaceous lipids. Δ6 desaturation, wax ester synthesis and squalene accumulation are the unique manifestations of sebaceous lipid metabolism. The importance of these unique sebaceous lipids for normal skin functions will be outlined.   Impairment of sebaceous lipid pathways in animal models resulted in severe skin and hair phenotypes. In addition essential fatty acids and their metabolites are fundamental for barrier function, healthy or atopic skin. New insights from clinical studies outline the importance of fatty acid metabolism for clear skin and normal barrier function. Understanding the roles of skin surface lipids is fundamental for decoding the skin physiology and homeostasis.

Gangliosides in the anti-pathological mechanisms of the gut

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Gangliosides (GG) are known to be biologically important molecules involved in cell differentiation, proliferation, neuritogenesis, growth, inhibition, signaling and apoptosis in animals and humans. Administration of GG to animals or humans prevents neuronal injuries from acute hypoxia and ischemic stroke, gut infection from LPS, and inflammatory signals. Human milk is enriched in GG, suggesting important roles in the neonatal intestine. It is not clear how dietary GG affects the neonatal development and gut protection from acute infection. Observations suggest that dietary GG alters the lipid profile of intestine and brain through the enrichment of GG in the serum. Dietary GG prevents inflammatory signals from acute exposure of the intestine to LPS by altering the composition of GG and the expression of caveolin protein in the lipid microdomains. Consumption of dietary GG also protects the degradation of a tight junction protein in response to acute LPS exposure. Infant bowel treated with GG is protected from inflammation during LPS exposure and hypoxia by reducing the production of nitric oxide (NO) and inflammatory mediators. These results underlie the importance of GG for early developmental and anti-pathological mechanisms.

Effects of fish oil supplementation on learning and behaviour in Indigenous children from remote community schools

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Background: Indigenous Australian children have significantly lower literacy and education outcomes than non-Indigenous children. They are also at risk for malnourishment, and Australian children generally are not consuming enough of the healthy food groups including omega-3 polyunsaturated fatty acids (n-3 PUFA). These are critical for healthy brain function and may assist with learning and behaviour.

Objective: To investigate the feasibility of administering fish oil supplements daily within the school environment in an open label pilot study.

Design: The study was conducted over 12 weeks in a remote Northern Territory (NT) school giving children 6 small fish oil capsules (providing 750mg long-chain n-3 PUFA) each school day. Assessments included reading, spelling, Ravens Coloured Matrices (non-verbal problem solving) and Draw-A-Person (DAP; non-verbal test of intelligence).

Outcomes: Forty seven children were recruited; the majority of the school population. Thirty seven children aged 5-14 (M=8.49, SD=2.29) took on average >3 capsules per school day over 12 weeks. Children were excluded due to problems swallowing the capsules, non-attendance/ leaving the school or too young for assessments (<5 years). We identified an appropriate assessment battery within this population and school environment, focusing on literacy and non-verbal cognition. Following initial disruption the daily supplementation and compliance recording went smoothly and teachers reported that it was worthwhile, with anecdotal reports of improved learning and behaviour. Linear mixed model analyses found improved age-adjusted scores on reading (p=.01), spelling (p<.01) and the Ravens (p<.01), indicating improvements beyond the expected age-related trajectory.

Conclusion: Fish oil supplementation was well received within the school environment and initial outcomes for learning and behaviour are encouraging. This study was followed up by an Australian Research Council funded placebo-controlled trial in four NT primary schools with over 300 children throughout the 2011 school year. Data collection will be completed in December and results analysed in 2012.

Omega-3 fatty acid supplementation rescues learning and memory deficits associated with Fetal Alcohol Spectrum Disorders

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When alcohol is consumed during pregnancy, the developing brain is significantly damaged. This damage can take the form of a number of disorders that are grouped under the term Fetal Alcohol Spectrum Disorder (FASD). One of the characteristic hallmarks of FASD is impairment in learning and memory processes. Experimentally we can study long-term potentiation (LTP), a biological model of learning and memory, in the dentate gyrus of the hippocampus. In this study we have observed deficits in LTP in adult males who have been exposed to alcohol prenatally. These deficits are accompanied by reductions in important neuronal antioxidants such as glutathione.

To try and ameliorate the deficits caused by prenatal alcohol exposure, we have given animals' access to a diet supplemented with omega-3 fatty acids. Omega-3 fatty acids are important for membrane fluidity and participate in many signaling cascades in the brain. We have found that supplementation can completely reverse the deficits in LTP and increase glutathione levels in alcohol exposed animals. These results indicate that omega-3 fatty acids may be an important addition to the diet of children suffering from FASD and may be able to "rescue" the deficits in learning and memory common with this disorder.

Further to this, we have determined that the deficit in LTP in alcohol exposed animals may be a direct cuase of glutathione depletion, as depleting glutathione in control animals produces a similar deficit in LTP to that observed in alcohol exposed animals. Future studies are aimed at determining the interaction between glutathione and LTP.

Bench to Bedside to Better Living: Our Journey from Basic Science to Clinical Trial Discovery Using Flaxseed as an Intervention for Cardiovascular Disease

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Dietary interventions are known to have significant potential as preventive medicine strategies in the fight against cardiovascular disease. Flaxseed (linseed) is a nutritional intervention that has been suggested to be of benefit against cardiovascular disease. It is one of the largest plant sources of alpha linolenic acid, an omega-3 polyunsaturated fatty acid. Polyunsaturated fatty acids have known cardioprotective actions. Flaxseed is also rich in antioxidants and fibre, two more components with documented cardiovascular effects. We have shown that flaxseed has potent anti-inflammatory effects, anti-atherogenic actions and anti-arrhythmic properties in animal models that appear important in the fight against heart disease. These data have led us into small human trials which then progressed into a major, year-long double blinded, placebo-controlled RCT in patients with cardiovascular disease. The talk will briefly describe these effects and our pathway to recent results in human trials.

Imbalances in 13C-DHA metabolism in the elderly and in APOE4 carriers

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Background: Docosahexaenoic acid (DHA) supplementation studies suggest that subtle imbalances in DHA metabolism occur with age and in apolipoprotein E epsilon 4 (ApoE4) carriers, and may contribute to increasing susceptibility to cognitive decline in the elderly. 13C-DHA is a promising tool to assess these imbalances in more detail.

Objective: To describe 13C-DHA metabolism in the elderly and in APOE4 carriers over 28 days.

Methods: In two independent studies, subjects received a single 50 mg oral dose of 13C-DHA and 13C distribution was followed in blood and breath over 28 days. In STUDY 1, we compared six young (26.8 ±

2.6 y old) and six elderly (76.5 ± 2.7 y old) whereas in STUDY 2, we compared 34 non-carriers of APOE4 to six carriers of APOE4.

Results: In both studies, plasma 13C-DHA peaked 4 to 6 h post-dose, reaching a minimum of 0.33 ± 0.16 nmol/ml of plasma in young participants and a maximum of 2.12 ± 0.99 nmol/ml of plasma in non-carriers of APOE4. In the elderly (STUDY 1), 13C-DHA remained transiently higher in the first 7 d of the follow-up, and apparent retro-conversion was 2 times higher than in the young. In APOE4 carriers (STUDY 2), plasma 13C-DHA was 50-75% lower in postprandial samples, whereas apparent retro-conversion was not different than in non-carriers. Cumulative beta-oxidation in the elderly was not different from in the young, whereas in APOE4 carriers, cumulative beta-oxidation was 2-3-fold higher at day 21 and 28. Whole body 13C-DHA half-life was ~21 d in APOE4 carriers and ~50 d in non-carriers of APOE4, and was not different between the young and elderly.

Conclusion: Using 13C-DHA, we demonstrate two types of imbalance in DHA metabolism in humans: (i) in the elderly, 13C-DHA was retained longer in the blood, whereas in APOE4 carriers, (ii) DHA undergoes more beta-oxidation than in non-carriers.

The role of omega-3 fatty acids in the treatment of parenteral nutrition associated liver disease

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OBJECTIVE(S): Parenteral nutrition-associated liver disease (PNALD) can be a lethal complication in children with short bowel syndrome (SBS). Intravenous fat emulsions (IFE) based on soybean oil administered with parenteral nutrition (PN) may contribute to its etiology. The objective was to determine the safety and efficacy of a fish oil-based IFE in the treatment of PNALD.

METHODS: We performed an open-labeled trial of a fish-oil IFE in 42 infants with SBS who developed cholestasis (serum direct bilirubin > 2 mg/dL) while receiving soybean IFE. Safety and efficacy outcomes were compared with those from a contemporary cohort of 49 infants with SBS and cholestasis whose PN course included soybean IFE only. The primary end-point was time to reversal of cholestasis (direct bilirubin ≤ 2 mg/dL).

RESULTS: Three deaths and 1 liver transplantation occurred in the fish oil cohort, compared to 12 deaths and 6 transplants in the controls (P=0.006). Among survivors not transplanted, cholestasis reversed while receiving PN in 19/38 patients in the fish oil cohort vs. 2/32 patients in the controls. Based on Cox models, subjects receiving fish oil-IFE experienced reversal of cholestasis 8.1 times faster (95% CI=1.9,35.5) than those receiving soybean IFE. The provision of fish oil IFE was not associated with hypertriglyceridemia, coagulopathy, essential fatty acid deficiency or growth delay. Moreover, hypertriglyceridemic events and abnormal INR levels were more common among controls.

CONCLUSIONS: Fish oil IFE is safe, may be effective in treating PNALD, and may reduce mortality and organ transplantation rates in children with SBS.

Metabolic engineering of very long chain polyunsaturated fatty acids in plants: accomplishment and challenge

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Very long chain polyunsaturated fatty acids (VLCPUFAs) such as arachidonic acid (ARA, 20:4-5,8,11,14), eicosapentaenoic acid (EPA, 20:5-5,8,11,14,17) and docosahexaenoic acid (22:6-4,7,10,13,16,19) are essential for human health and well-being. The current sources of these fatty acids are fish from ocean which is limited, overexploited and not sustainable. In the past few years, considerable effort has been made in identifying genes from microalgae and fungi involved in the biosynthesis of VLCPUFAs and expressing these genes in plants for the transgenic production of these fatty acids as an alternative source. This presentation will present our work in metabolic engineering of VLCPUFAs in oilseed crops made over the past few years and discuss current challenge and possible strategy in production of high levels of these fatty acids in plants.

Breaking science in linoleic acid (LA) intervention trials

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Randomized controlled trials (RCT) with provision of LA-selective study oils offer a rare opportunity to evaluate the specific effects of increasing the n-6 PUFA LA, without protential confounding from n-3 PUFAs. Here we present: (1) outcome data from an RCT that evaluated the effects of selectively increasing LA in place of saturated fats, and (2) results of the first complete risk/benefit assessment of the CVD effects of selectively increasing LA, incorporating all known RCT datasets.

Branched Chain Fatty Acids Reduce the Incidence of Necrotizing Enterocolitis and Alter Gastrointestinal Microbiota in a Neonatal Rat Model

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Background & Objective: Branched chain fatty acids (BCFA) are found in the normal term human newborn gut, deposited as major components of vernix caseosa ingested during late fetal life. We hypothesized that premature infant lack of GI BCFA exposure is associated with the risk for necrotizing enterocolitis (NEC) and with their microbiota in an animal model.

Procedure: Premature rat pups were assigned to one of three diets: dam-fed (DF), rat formula (Control), and rat formula with 20%w/w BCFA (BCFA). All groups were exposed to NEC inducing conditions. Cecal microbiota, intestinal injury, cytokine and mucin gene expression, and BCFA uptake in ileum phospholipids (PL), serum and liver were evaluated.

Results: NEC incidence was reduced by 56% in the BCFA group compared to the Control group; BCFA-fed pups had a greater relative abundance of BCFA-associated Bacillus subtilis and Pseudomonas aeruginosa compared to Controls, and B. subtilis levels were greater in healthy pups compared to sick pups. BCFA were selectively incorporated into ileal PL, serum and liver tissue and IL-10 expression increased in the BCFA group compared to Controls.

Conclusion: BCFA reduced NEC incidence, and altered microbiota composition. BCFA were also incorporated into pup ileum and were associated with enhanced IL-10 expression.

A lipid pathway for ligand binding is necessary for a cannabinoid G protein-coupled receptor

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Isothiocyanate covalent labeling studies have suggested that a classical cannabinoid, (-)-7'-isothiocyanato-11-hydroxy-1',1'dimethylheptyl-hexahydrocannabinol (AM841), enters the cannabinoid CB2 receptor via the lipid bilayer (Pei et al. Chem Biol 15: 1207, 2008). However, the sequence of steps involved in such a lipid pathway entry has not yet been elucidated. In work to be presented, we tested the hypothesis that the endogenous cannabinoid, sn-2-arachidonylglycerol (2-AG) attains access to the CB2 receptor via the lipid bilayer. To this end, we employed microsecond time scale all-atom molecular dynamics (MD) simulations of the interaction of 2-AG with CB2 via a palmitoyl-oleoyl-phosphatidylcholine (POPC) lipid bilayer. Results suggested that (1) 2-AG first partitions out of bulk lipid at the TMH6/7 interface; (2) 2-AG then enters the CB2 receptor binding pocket by passing between TMH6/7; (3) the entrance of the 2-AG head group into the CB2 binding pocket is sufficient to trigger breaking of the intracellular (IC) TMH3/TMH6 ionic lock and the movement of the TMH6 IC end away from TMH3; (4) subsequent to protonation at D3.49/D6.30, further 2-AG entry into the ligand binding pocket results in both a W6.48 toggle switch change and large influx of water. Interestingly, even in this fully activated CB2/2-AG complex, part of the 2-AG acyl tail has not yet entered the binding pocket. In additional POPC bilayer simulations of CB2 (activated by 2-AG, see (3) above) in complex with Gi protein, insertion of the

G-alpha-i C-terminus into the exposed CB2 IC domains results in the complete entrance of 2-AG. To our knowledge, this is the first demonstration via unbiased molecular dynamics (MD) that a ligand can access the binding pocket of a Class A GPCR via the lipid bilayer and the first demonstration via MD of GPCR activation triggered by a ligand binding event. [Support: NIDA RO1 DA003934 and KO5 DA021358]

The importance of Omega-3 LC-PUFA for children's behaviour and learning: current evidence and implications for research and practice

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Relatively low dietary intakes of omega-3 LC-PUFA are thought to contribute to a wide variety of physical and mental health problems in adults. In early life, the essentiality of these fatty acids for normal brain development is well established, and international dietary recommendations for pregnancy and infancy acknowledge the importance of omega-3 LC-PUFA, and DHA in particular, to support normal visual and cognitive development.

Much less is currently known about optimal intakes of omega-3 LC-PUFA in older children and adolescents, and how any deficiencies may affect brain development and functioning during these stages of life. Evidence from clinical trials does indicate that dietary supplementation with omega-3 LC-PUFA may improve at least some aspects of child behavior and learning. To date, however, most such studies have involved children with neurodevelopmental disorders such as attention-deficit/hyperactivity disorder (ADHD) or developmental coordination disorder (DCD), making it difficult to know whether similar benefits from supplementation might extend to the general child population. Most trials in this area have also been small, with considerable differences between populations studied, treatment formulations used, and outcomes assessed.

Current evidence in this area will be critically reviewed here, and the implications of this for both research and clinical practice will be considered. Outline results will also be presented from a newly completed randomised controlled trial - the DHA Oxford Learning and Behaviour (DOLAB) study - investigating the effects of supplementation with DHA on reading, working memory and behaviour in healthy children from mainstream schools in the UK.

Lipoprotein Lipase-Master Controller of Fatty Acid Delivery to the Heart after Diabetes

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Heart has a limited potential to synthesize fatty acid (FA) and therefore FA is supplied from several sources: lipolysis of endogenous cardiac triglyceride (TG) stores, or from exogenous sources in the blood. Lipoprotein lipase (LPL), synthesized in cardiomyocytes, catalyzes the breakdown of the TG component of lipoproteins to provide FA to the heart. It is the vascular endothelial-bound LPL that determines the rate of plasma TG clearance and hence, it is also called heparin releasable (HR) "functional" LPL. Functional LPL is regulated by numerous dietary and hormonal factors, and is sensitive to pathophysiological alterations like those observed during diabetes. In this condition, absolute or relative lack of insulin impairs cardiac glucose transport and oxidation, resulting in FA becoming the preferred means of energy supply. To make available this increased requirement of the heart for FA, diabetic heart upregulates its luminal LPL activity by posttranslational mechanisms. Chronically elevated cardiac LPL can result in abnormal FA supply and utilization by the heart tissue that could potentially initiate and sustain cardiac dysfunction during diabetes. In this talk, the regulation of cardiac LPL will be discussed, and an attempt will be made to piece together how early metabolic changes could instigate diabetic heart disease.

Benefits of the Mediterranean diet patterns on CVD risk: the PREDIMED study.

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The Mediterranean diet (MeDiet) is characterized by a high intake of plant-derived foods; a moderate intake of fish and wine; and a low intake of dairy products, meat and meat products, and sweets. The MeDiet is a high-fat, high-monounsaturated fatty acid (MUFA) food pattern because olive oil is used abundantly as culinary fat. Epidemiological evidence suggests an inverse association between MeDiet adherence and cardiovascular disease (CVD). In small trials, MeDiets have shown beneficial effects on risk factors and surrogate markers of CVD, which adds biological plausibility to epidemiological evidence. The PREDIMED study is a randomized, 5-y clinical trial conducted in Spain to assess the effect of MeDiets on incident CVD. We randomly assigned 7447 persons (mean age, 67 y; 43% men) at high cardiovascular risk but no CVD at enrolment to one of three diets: MeDiet supplemented with extra-virgin olive oil (EVOO), MeDiet supplemented with mixed nuts, or Control diet (advice to reduce all dietary fat). Participants received quarterly individual and group sessions and, free provision of either EVOO (one liter/week), nuts (15 g walnuts, 7.5 g almonds and 7.5 g hazelnuts/day), or small non-food gifts. Intervention resulted in MUFA and polyunsaturated fatty acid (PUFA) intake changes (% energy) of 2,5, 1.3, and -0.5 and 0, 1.3, and -0.7 for MeDiet+EVOO, MeDiet+nuts and Control diet groups, respectively. Saturated fat was similarly reduced by ≈1% in all groups. Results showed that, compared with the Control diet, the MeDiet+nuts reduced the 1-y prevalence of metabolic syndrome by 14% and the two MeDiets reduced the 4-y incidence of diabetes by 52%. The MeDiets also had salutary effects on blood lipids, insulin resistance, blood pressure, and oxidation and inflammation biomarkers. In conclusion MeDiets supplemented with foods rich in MUFA or PUFA but also in polyphenols and other bioactive molecules are beneficial for CVD risk.

Interplay Between Caveolin-1, Mitochondrial-Enriched Membranes and Lipid Metabolism

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Caveolins (CAV) are essential components of caveolae; cholesterol-enriched membrane microdomains of most mammalian cells. CAV1, the most common isoform of CAVs, has a critical role in caveolae assembly. Besides, CAV1 is likely to have additional intracellular functions as it is not restricted to the plasma membrane and has been found in several intracellular organelles such as lipid droplets (LDs), endoplasmic reticulum (ER) and mitochondrial-associated membranes (MAMs). In humans, CAV1 mutations result in lipodystrophies and the CAV1 gene-disrupted mice (CAV1-/-) display a phenotype of partial lipodystrophy and resistance to obesity. In the liver, the CAV1-/- mice show an important intracellular lipid imbalance, decreased formation of LDs and a cholesterol promoted mitochondrial dysfunction, suggesting a pivotal role of CAV1 in hepatic lipid metabolism. Such a lipid imbalance predisposes CAV1-/- cells and animals to diseases such as steatohepatitis and reduces liver regeneration after partial hepatectomy. However, the true functional relevance of CAV1 in the regulation of the intracellular lipid fluxes and associated diseases remains mostly unknown. Given that several enzymes involved in lipid metabolism are reported to locate at hepatocyte MAMs, we hypothesized that lack of functional CAV1 in hepatic MAMs would lead to impaired interorganelle lipid transport, especially between ER and mitochondria. To this end, we (i) adapted a protocol of isolation of MAMs and mitochondria from animal tissues and (ii) designed a proteomics and lipidomics analysis of MAMs and mitochondria of both wild type and CAV1-/- mice. We have obtained evidence that CAV1 is significantly enriched in MAMs

when compared to the bulk of the ER. Proteomics and lipidomics analysis of the cellular fractions is currently underway.

Plasma long-chain omega-3 fatty acids and atrophy of the medial temporal lobe: A longitudinal study

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Objective: EPA and DHA are potential candidates for interventions to delay Alzheimer's disease (AD), but evidence from clinical studies is mixed. The association between EPA and DHA status and MRI biomarkers of neurodegeneration in AD has never been studied. We aimed at determining whether plasma levels of EPA and/or DHA predict atrophy of medial temporal lobe (MTL) grey matter regions in older subjects.

Methods: 281 community dwellers from the Three-City Study, aged 65 years or older, had plasma fatty acid measurements at baseline and underwent MRI examinations at baseline and at 4 y. We studied the association between plasma EPA and DHA and MTL grey matter volume change at 4 y.

Results: Higher plasma EPA, but not DHA, was associated with less grey matter atrophy of the right hippocampal/parahippocampal area and of the right amygdala ($P<0.05$, family-wise-error corrected). Based on a mean right amygdala volume loss of 6.0 mm3/y (0.6%), a 1 standard deviation higher plasma EPA (+0.64% of total plasma fatty acids) at baseline was related to a 1.4 mm3 smaller grey matter loss/y in the right amygdala. Higher atrophy of the right amygdala was associated with greater 4 y decline in semantic memory performances and more depressive symptoms.

Conclusion: The amygdala, which develops neuropathology in the early stage of AD and is involved in the pathogenesis of depression, may be a major brain structure involved in the association between EPA and cognitive decline and depressive symptoms.

Does palmitic acid in the sn-2 position have different effects on cardiovascular risk from that in the sn-1 and sn-3 positions?

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Palmitic acid in palm olein (PO) is present in the sn-1 and sn-3 positions, whereas in animal fats it is in the sn-2 position. Interesterified palm oil is used in the manufacture of high melting point fats which are required for certain food applications (bakery goods, margarine). It has been suggested that the process of interesterification might adversely effect lipid metabolism and glucose homeostasis and increase risk of cardiovascular disease. To test this hypothesis we compared native PO (IV 56, 16:0 9.2 mol% at sn-2) with PO that had been randomly interesterified (IPO, IV 56, 16:0 39.1 mol% at sn-2) in human volunteer studies using a cross-over design. We first compared the fats (50g) in a postprandial test meal study in London and Maastricht in 25 men and 25 women. IPO resulted in a slower increase in postprandial lipemia following the test meal. The proportion of palmitic acid in the sn-2 position of chylomicron triacylcerols was 42 mol% in the IPO vs 24% in PO. There was a reduced release of glucose-dependent insulinotropic polypeptide ($P<0.001$) following IPO vs native PO but this did not influence insulin release or glucose homeostasis. The second study conducted in Malaysia involved exchanging two-thirds of the dietary fat intake provided either as PO or IPO. Participants were provided with food 5 days a week in a canteen and given the test oil to use for home cooking at weekends. Each dietary period

lasted 6 weeks and at the end of each period fasting and postprandial blood samples were collected. The initial results on 41 participants indicate no differences between PO and IPO on fasting plasma lipid profile, insulin release or indices of insulin sensitivity. In conclusion, our results indicate no evidence of adverse effects on cardiovascular risk factors of interesterification of palm olein.

DHA supplementation prevents age-related functional losses and A2E accumulation in the retina

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Purpose: With age, retina function progressively declines and A2E, a constituent of the toxin lipofuscin, accumulates in retinal pigment epithelial (RPE) cells. Both events are typically exacerbated in age-related retina diseases. We studied the effect of dietary DHA (C22:6n-3) supplementation on these events, using a transgenic mouse model (mutant human ELOVL4; E4) displaying age-related retina dysfunction and RPE A2E accumulation.

Methods: Retina function was assessed with the electroretinogram (ERG) and A2E levels were measured in E4 and wildtype (WT) mice. Dietary DHA was manipulated from 1-3, 1-6, 6-12, and 12-18 months (mo): 1.0% DHA over total fat (E4+, WT+) or similar diet without DHA (E4-, WT-).

Results: Increased omega-3/6 ratios (DHA/arachidonic acid) in E4+ and WT+ retinas were confirmed for the 1-3mo and 1-6mo trials. While 1-3mo intervention had no effects, when prolonged to 1-6mo, RPE function (ERG c-wave) was preserved in E4+ and WT+. Intervention from 6-12mo led to maintained outer and inner retina function (ERG a- and b-wave) in E4+. At 12-18mo, a similar beneficial effect on retina function occurred in WT+; A2E levels were reduced in E4+ and WT+.

Conclusion: DHA supplementation was associated with: 1) preserved retina function at mid-degenerative stages in E4 mice; 2) prevention of age-related functional losses in WT mice; and 3) reduced A2E levels in E4 and WT mice at the oldest age examined. These findings imply that dietary DHA could have broad preventative therapeutic applications (acting on pathological and normal age-related ocular processes).

Impact of early nutrition on infectious and allergic symptoms and diseases in the first year of life

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Background – Inclusion of appropriate levels of docosahexaenoic acid (DHA) and arachidonic acid (ARA) in infant formulas may protect against infectious and allergic diseases.

Objective – To assess the effect of DHA/ARA intake in early infancy on the incidence of infectious and allergic events in the first year of life.

Design – In this multi-center, observational, prospective study, infants received either a marketed cow's milk-based formula with DHA and ARA (0.32% and 0.64% of total fatty acids, respectively) (DHA/ARA, n=233) or the same formula without DHA/ARA (Control, n=92). Anthropometrics were assessed at enrollment (<60 days of age) through 12 months of age. At each study visit parents completed a questionnaire to record the incidence of infectious and allergic events.

Outcomes – Study completion did not differ between groups [Control, n=78 (85%); DHA/ARA, n=204 (88%)]. There were no significant group differences in weight-, length-, and head-circumference-for-age z-scores (relative to US National Center for Health Statistics reference population) for males at any time point. Group z-scores for females differed for weight-for-age at 6 (Control = -0.0±0.1, DHA/ARA = 0.4±0.1; p=0.011), 9 (Control = -0.2±0.1, DHA/ARA = 0.3±0.1; p=0.003), and 12 (Control = -0.3±0.2, DHA/ARA = 0.1±0.1; p=0.033) months of age. However, these differences were not clinically relevant (all z-scores were within the normal range). The DHA/ARA group had significantly lower odds (OR, 95% confidence interval [CI]) of having an increased number of episodes of nasal congestion (0.45, 0.29-0.69; P<0.001), cough (0.47, 0.30-0.74; P=0.001), croup (0.23, 0.05-0.97; P=0.045), diarrhea (0.50, 0.27-0.90; P=0.021) and adjusted OR for bronchiolitis (0.43, 0.24-0.78; P=0.005), bronchitis (0.41, 0.23-0.71; P=0.001), wheezing (0.50, 0.27-0.92; P=0.027), and eczema (0.57, 0.33-0.98; P=0.043). No group differences were detected for otitis media.

Conclusion – Early nutrition with DHA/ARA supplementation was associated with reduced incidence of infectious and allergic symptoms and diseases in the first year of life.

FADS2 gene variant influences the proportion of DHA in human milk

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FADS1 and FADS2 encode the rate-limiting enzymes responsible for converting alpha-linolenic acid to docosahexaenoic acid (DHA). Caspi et al. (2007) found breastfeeding confers an IQ-point advantage only to children carrying the major allele for a single nucleotide polymorphism (SNP) in FADS2. Since this time, it has been demonstrated that SNPs in FADS1/2 influence the proportion of blood lipid and breast milk DHA. However, previous studies have not controlled for maternal DHA status to isolate the effect of polymorphisms in the FADS gene cluster. The objective of this study was to determine if SNPs in maternal FADS1 rs174553 and FADS2 rs174575 influence the proportion of DHA in breast milk, after controlling for the proportion of DHA in maternal red blood cells (RBCs). The study population consisted of a subset of 117 women enrolled in an NICHD-funded Phase III clinical trial (NCT00266825). Women provided blood and breast milk samples the morning after and approximately six weeks following parturition, respectively. Milk total lipids and RBC phospholipids were transmethylated with boron trifluoride-methanol, and the resulting fatty acid methyl esters were quantified by gas liquid chromatography in comparison with weighed standards. Genomic DNA was extracted from buccal collection brushes, and genotyping performed with made-to-order TaqMan SNP Genotyping Assays using real-time PCR. A first-order linear regression model was used to determine the main effect of FADS minor alleles on breast milk DHA, controlling for the proportion of DHA in maternal RBCs. Women homozygous for FADS2 minor alleles had a significantly lower proportion of DHA in their breast milk ($p = 0.042$) than major allele carriers. The results of the present study support the hypothesis that polymorphisms in FADS2 limit the incorporation of DHA in breast milk and may account for the observed IQ advantage among major-allele carriers. (Supported by the National Institutes of Health: HD047315.)

Fish oil increases B cell lipid microdomain size and membrane order accompanied by immunosuppressive effects on antigen presentation

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Fish oil (FO) targets lipid microdomain organization to suppress T cell and macrophage function; however, little is known about this relationship with B cells, especially at the animal level. Therefore, we tested the hypothesis that FO would disrupt lipid raft microdomain organization of B cells accompanied by immunosuppressive effects. We first measured how FO, administered to mice at a dose modeling human intake, disrupted B cell lipid rafts induced by cross-linking GM1 molecules. Total internal reflection fluorescence and polarization imaging studies respectively revealed FO, relative to controls, increased the size of B cell rafts and increased membrane order upon cross-linking rafts relative to no cross-linking. We then conducted experiments to determine which bioactive fatty acid in FO was responsible for disrupting lipid rafts. A combination of ex vivo biochemical studies, cell culture experiments, and NMR measurements using model membranes revealed that docosahexaenoic (DHA), but not eicosapentaenoic (EPA) acid, disrupted membrane raft size and order. Finally, we tested the hypothesis that disrupting rafts with FO would suppress B cell mediated antigen presentation to T cells, a therapeutic target in the inflammatory response. Indeed, FO suppressed the ability of B cells to stimulate transgenic naïve CD4+ T cells, as measured by cytokine secretion. Altogether, studies with B cells suggest a model in which FO increases lipid microdomain size and membrane order to exert immunosuppressive effects; furthermore, the results highlighted differences in EPA and DHA bioactivity.

Disruption of lipoprotein oxylipins in metabolic syndrome and partial correction by omega-3 fatty acids

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Introduction: The oxygenation of polyunsaturated fatty acids (PUFA) produces eicosanoids and other oxylipin signaling molecules which mediate physiologic functions ranging from activation and recruitment of immune cells to regulation of endothelial cell membrane polarity. Since circulating lipoproteins carry acylated oxylipins, we tested whether lipoprotein oxylipins are disrupted in disease and whether omega-3 fatty acids would correct any disruption.

Design and methods: Lipoproteins from optimally healthy controls (N=14) and metabolic syndrome (MetSyn; N=30) subjects were compared. MetSyn subjects were then randomized into placebo (n=13) or prescription omega-3 ethyl ester (P-OM3; 4g/day; n=17) groups for 8 weeks of treatment. Oxylipins derived from 18-, 20- and 22-carbon PUFAs were measured as epoxides, vicinal diols, mid-chain alcohols, ketones, leukotrienes, and prostanoids were measured by LC MS and normalized to lipoprotein phospholipid content. Cluster analysis was performed for dimension reduction followed by testing for group differences.

Results: Specific, not all, oxylipins were disrupted by disease or corrected by treatment: of 10 clusters identified, two were different between healthy controls and metabolic syndrome subjects. The first was comprised of HDL oxylipins (p = 0.01) as arachidonate lipoxygenase metabolites plus PGF2-alpha. The second contained nearly all VLDL linoleic and alpha-linolenic acid oxylipins, DHA epoxides, and arachidonate diols (P<0.0001). P-OM3 treatment, changed the arachidonate HDL oxylipins (p= 0.02) and globally increased omega-3 oxylipins across VLDL, LDL and HDL. Additionally, a third cluster of arachidonate lipoxygenase oxylipins in LDL were reduced by treatment (p= 0.01). In each case, treatment corrected levels towards those in healthy controls, hypercorrecting them in the case of omega-3 metabolites.

Conclusion: Metabolic Syndrome is characterized by changes in specific lipoprotein oxylipins. P OM3 treatment corrects or hypercorrects these changes and are likely tod improve lipoprotein signaling.

Characterizing the ganglioside content of bovine milk using LC/MS

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Gangliosides (GG), key cell membrane components that are involved in gastrointestinal health and neural development, represent an interesting and challenging analytical target. Mass spectrometry (MS), combined with liquid chromatography (LC), is an emerging tool in gangliosides analysis due to its speed, sensitivity, and specificity. An LC/MS based assay is presented using reversed phase chromatography combined with a triple quadrupole MS to compare the GG profile in fresh milk and buttermilk powder.

Samples were prepared for LC/MS analysis using a modified folch extraction. Due to the high sensitivity afforded by an LC/MS based assay, a small-scale folch extraction was developed. The original protocol required 5 ml of sample, 2 days, and generated 200 ml of solvent waste. The optimized method requires only 100 µl of sample, 30 minutes, and generated 1.5 ml of solvent waste. Following extraction, the samples were dried under nitrogen and dissolved in the LC mobile phase.

For detection of gangliosides species, the triple quad MS was operated in multiple reaction monitoring mode. Since mono- and disialogangliosides are expected to be the most abundant GGs in bovine milk, a theoretical mass database was created for these species with variations in carbon chain length and degree of saturation in the ceramide moiety. Whole milk and buttermilk powder was screened by LC/MS against this database. In total, the samples were screened against 468 different gangliosides species in 3 hours. Buttermilk powder and fresh milk had a very similar GG profile. Consistent with the literature, GD3 was found to be the most abundant GG class, in addition to a small amount of GM3 and GT3

species. Assuming that the ceramide consists of shingosine (d18:1) or dihydroshpingosine (d18:0), the most abundant fatty acid side chains were C24:0, C22:0, and C20:0 for all GG classes.

Docosahexaenoic acid and curcumin induce synergistic cellular and molecular effects in breast cancer cells

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Docosahexaenoic acid (DHA) and curcumin (CCM) are dietary compounds known to inhibit breast cancer cell proliferation. We investigated if a combination of these compounds exerts a synergistic effect in breast cell lines. Dose response curves for DHA and CCM were generated for five breast cell lines. Effects of the DHA+CCM combination on cell proliferation were evaluated using varying concentrations, at a fixed ratio, of DHA and CCM based on their individual ED50 values. Cell molecular network responses were investigated through whole genome microarray analysis of transcript level changes. Gene expression results were validated by RT-PCR, and western blot analysis was performed for potential cellular mediators.   DHA+CCM had an anti-proliferative effect in SK-BR-3, MDA-MB-231, MDA-MB-361, MCF7 and MCF10AT cells. The effect was synergistic for SK-BR-3 (ER- PR- Her2+) relative to the two compounds individually. CCM+DHA triggered transcript-level responses, in disease-relevant functional categories, that were largely non-overlapping with changes caused by DHA or CCM individually. Genes involved in cell cycle arrest, apoptosis, inhibition of metastasis, and cell adhesion were upregulated, whereas genes involved in cancer development and progression, metastasis, and cell cycle progression were down regulated. Most notable, a set of cytochrome P450 (CYP450) and SERPINB5 transcript levels were upregulated 20- to 100-fold, relative to untreated cells, by DHA+CCM treatment. Cellular pools of PPARγ and phospho-p53 were increased by DHA+CCM relative to either compound alone. DHA enhanced cellular uptake of CCM in SK-BR-3 cells without significantly enhancing CCM uptake in other cell lines. The combination of DHA and CCM is potentially a dietary supplemental treatment for some breast cancers, likely dependent upon molecular phenotype. DHA enhancement of cellular curcumin uptake is one potential mechanism for the observed synergy in SK-BR-3 cells; however, transcriptomic data show that the anti-proliferation synergy accompanies many signaling events unique to the combined presence of the two compounds.

Docosapentaenoic Acid Supplementation Study in Humans

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Despite the detailed knowledge of the absorption and incorporation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in humans, very little is known about DPAn-3. Therefore, the aim of this randomised crossover double blind study was to investigate the uptake and incorporation of pure DPAn-3 into human plasma lipid fractions. Ten female subjects received 9 g of pure EPA or pure DPAn-3 over a 7-day period. The placebo treatment was olive oil. Fasting blood samples were collected at days zero, four and seven, following which the plasma was separated and used for fatty acid analysis. Supplementation with EPA significantly increased the proportion of EPA in the cholesterol ester (CE) and phospholipid (PL) fractions (d4 and d7), with no changes recorded in the proportions of DPAn-3 or DHA in any lipid fraction in this group. Supplementation with DPAn-3 resulted in significantly increased proportions of DPAn-3 in the PL fraction (d4 and d7), and in the TAG and CE fractions (d4 only). In the DPAn-3 group, there were also significantly increased proportions of EPA in TAG and CE (at d4), and of DHA in TAG (d4 and d7) and PL (d4). These results showed that EPA and DPAn-3 are incorporated in different and specific patterns in plasma lipids. The results of this short-term study also suggested the existence of retro-conversion of DPAn-3 to EPA and bioconversion of DPAn-3 to DHA.

Human endotoxemia as a research model for studying the effects of fatty acids on induced inflammation

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Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may blunt the response to an inflammatory challenge and/or enhance its resolution in humans; however, research models to examine this question face significant challenges concerning safety, consistency, and efficacy. Our studies are utilizing an in vivo inflammatory challenge with intravenous administration of low dose endotoxin (0.6 ng/kg). A saline placebo-controlled crossover preliminary study was conducted to characterize the safety and efficacy of this research model in six healthy men. An additional research aim was to characterize the timing of inflammation resolution. Participants were randomized to a 2-period, saline placebo-controlled, crossover study design with 6 weeks between testing sessions. Blood was sampled at a pre-injection baseline and 1, 2, 3, 4, 6, 12, 24, 48, 72, and 120 hours post-injection. All meals were standardized and controlled for the first 12 hours. A mixed linear model demonstrated a treatment by time interaction for TNF-α, IL-6, and hs-CRP ($p < 0.0001$ for all). Post hoc analyses (adjusted for multiple comparisons) indicated several time specific differences between treatments ($p < 0.05$). TNF-α increased versus placebo at 1, 2, 3, and 4 hours post-injection by 19, 27, 13, and 6.2 pg/mL, respectively. Similarly, IL-6 increased at 2, 3, and 4 hours versus placebo by 21, 23, and 14 pg/mL, respectively. Serum hs-CRP increased at 12, 24, 48, and 72 hours versus placebo by 8.6, 16, 7.5, and 3.4 mg/L, respectively. Symptoms were absent or mild in nature (e.g. transient headache or malaise) and corresponded with peak inflammatory cytokine (IL-6, TNF-α) responses. Our findings demonstrate the safety and efficacy of the low dose endotoxemia model for inducing acute systemic inflammation. The endotoxemia model may serve as a useful research tool for characterizing the effects of fatty acid interventions on the human systemic inflammatory response and its resolution.

The emerging evidence for the interactions between n-3 fatty acids and iron

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Iron deficiency (ID) is the most common nutrient deficiency in the world, while populations with low fish and seafood intakes and a high use of vegetable oils rich in n-6 fatty acids (FA) are at risk of inadequate n-3 FA intakes. Both iron and n-3 FA are essential nutrients for normal brain development and immune function. Deficiencies of iron and n-3 FA (n-3 FAD) may interact directly via Fe-dependent hepatic desaturases and/or via iron-dependent.

Several animal studies and one study in South African school children have linked ID to alterations in lipid metabolism and tissue FA profiles. Conversely, changes in membrane properties with decreasing DHA contents may reduce activity of embedded receptors involved in Fe absorption and uptake into cells.

In a depletion rat study, we recently found that total phospholipid EPA and DHA contents were sharply reduced, while ALA was increased in RBCs of rats receiving an ID diet for 5 weeks. We also found significant effects of ID on n-3 and n-6 FA contents in rat brain. In contrast, n-3 FAD significantly lowered Fe concentrations in rat hippocampus and cerebellum. In a repletion study, we showed that DHA contents of several brain regions were higher when DHA/EPA was fed in combination with Fe than in rats receiving DHA/EPA alone, and repletion of brain Fe concentrations was more effective when Fe was fed in combination with DHA/EPA. Besides the direct interactions, we found that ID and n-3 FAD impair brain development and function through shared mechanisms.

Nutrient deficiencies seldom occur in isolation in humans, and the work of our lab highlights the importance of investigating the interactions of common deficiencies, particularly during critical periods in brain development and growth.

FADS1 FADS2 Gene Cluster, PUFA Intake and Blood Lipids in Children. Results from the GINIplus and LISAplus Studies.

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Background: Elevated cholesterol levels in children can be a risk factor for cardiovascular diseases in later life. In adults, it has been shown that blood lipid levels are strongly influenced by polymorphisms in the fatty acid desaturase (FADS) gene cluster in addition to nutritional and other exogenous and endogenous determinants. Our aim was to investigate whether lipid levels are determined by the FADS genotype already in children and whether this association interacts with dietary intake of n-3 fatty acids.

Methods: The analysis was based on data of 2006 children from two German prospective birth cohort studies. Total cholesterol, HDL, LDL and triglycerides were measured at 10 years of age. Six single nucleotide polymorphisms (SNPs) of the FADS gene cluster were genotyped. Dietary n-3 fatty acid intake was assessed by food frequency questionnaire. Linear regression modeling was used to assess the association between lipid levels, n-3 fatty acid intake and FADS genotype.

Results: Individuals carrying the homozygous minor allele had lower levels of total cholesterol [means ratio (MR) ranging from 0.96 (p=0.0093) to 0.98 (p=0.2949), depending on SNP's] and LDL [MR between 0.94 (p=0.0179) and 0.97 (p=0.2963)] compared to homozygous major allele carriers. Carriers of the heterozygous allele showed lower HDL levels [β between -0.04 (p=0.0074) to -0.01 (p=0.3318)] and higher triglyceride levels compared to homozygous major allele carriers [MR ranging from 1.06 (p=0.0065) to 1.07 (p=0.0028)]. A higher n-3 PUFA intake was associated with higher concentrations of total cholesterol, LDL, HDL and lower triglyceride levels, but these associations did not interact with the FADS1 FADS2 genotype.

Conclusion: Total cholesterol, HDL, LDL and triglyceride concentrations are determined by the FADS1 FADS2 genotype already in 10 year old children. Genetically determined blood lipid levels during childhood might differentially predispose individuals to the development of cardiovascular diseases later in life.

DHA supplementation improved memory and speed of memory in healthy young adults

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Background: Docosahexaenoic acid (DHA), a long chain omega (n)-3 fatty acid, is important for brain structure and function and is dependent on dietary intakes. Individuals following diets low in n-3 may cognitively benefit from increased DHA intake.

Objective: To investigate whether a high DHA supplement improves cognitive performance in healthy young adults.

Design: Healthy adults (n=176, 18-45 years, non-smoking, low intake of long chain n-3) completed a 6-month randomised placebo controlled double blind trial. Subjects were matched for age and gender and randomly assigned to either DHA (1.16 g DHA/day) or placebo. Cognitive performance was assessed using a computerised cognitive test battery. Z-scores were calculated and the different tests clustered into cognitive domains: episodic memory, working memory, attention, speed of memory and working memory

and attention. Intention-to-treat analysis was performed using ANCOVA (controlling for baseline and education) and adding gender as a factor.

Outcomes: Erythrocyte DHA levels increased significantly in the DHA group compared to the placebo group (mean (95% CI) from 5.28 to 7.87% vs. 5.06 to 5.01% respectively, P<0.001). Memory and working memory improved with DHA compared to placebo in women (mean (95% CI) difference 0.25 (0.05, 0.45) SD, P=0.01; 0.19 (0.01, 0.36) SD, P=0.04, respectively) but not in men. Speed of working memory improved with DHA compared to placebo in men (reaction time (RT) -0.56 (-0.90, -0.21) SD, P=0.002). Although the speed of memory domain failed to reach significance between treatments (P=0.07), speed of delayed word recognition improved in women (RT -0.34 (-0.59, -0.08) SD, P=0.01). Attention was not affected.

Conclusion: DHA supplementation improved memory and speed of memory in healthy young adults whose habitual diet was low in DHA. DHA affected the memory domains differently in men and women with memory and speed of long-term memory improving only in women and speed of working memory improving in men.

Trial registration: ACTRN12610000212055.

Mind-Body Interface: Polyunsaturated fatty acids and somatic symptoms in major depressive disorder

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Background. Lower n-3 polyunsaturated fatty acids (n-3 or omega-3 PUFAs) levels and genetic variations on their metabolic enzymes of PUFA metabolic enzymes, phospholipase A2 (PLA2) and cyclo-oxygenase-2 (COX2), have been found to be associated with the risk of depression (1-4). In this study, we aimed to examine specific roles of n-3 PUFAs, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and the polymorphisms on PLA2 and COX2 in different clusters of depressive symptoms.

Methods. Patients with major depressive disorders (n=122) and their healthy controlled subjects (n=122) were assessed to examine the effects of PUFA levels and single nucleotide polymorphisms (SNPs) of PLA2 BanI and COX2 rs4648308 genes on the development of major depression and on specific clusters of depressive symptoms.

Results. Patients with major depressive disorders had a significant lower level of EPA (p=0.03) and a trend of lower level of DHA (p=0.08). The COX2 rs4648308 AG genotype was associated with a higher risk of major depression (p=0.006; odds ratio=2.36, 95% CI=1.27-4.40), while the PLA2 BanI GG genotype had a borderline effect (p=0.06; odds ratio=1.81, 95% CI=0.87-3.79). The "at risk" COX2 polymorphism was associated with more somatic symptoms (p=0.003) and lower DHA (p=0.002), and the "at risk" PLA2 polymorphism was associated with more somatic symptoms (p=0.025). In addition, lower EPA and DHA levels were both significantly correlated with more somatic symptoms in patients with depression.

Conclusions. Genetic variations in the COX2 and PLA2 genes have effects on depression and somatic features, possibly by affecting the levels of EPA and DHA. N-3 PUFAs may be a potential biomarker to understand clinical subtypes of depression (1).

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Does interesterification as a fat modification tool impact nutritional outcomes?: A review of the evidence

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The imminent exit of trans fatty acids from the global food supply demands newer sources of solid fats. Currently available options include GMO oilseed varieties with higher saturation or modified fats through chemical or enzymatic interesterification (IE). IE primarily increases melting characteristics through altered triacylyglyceride (TAG) structures but without accompanying fatty acid changes. Subjecting liquid

oils to IE result in only minor physico-chemical alterations, largely inadequate for imparting solid fat functionalities. Blending these oils with solid fats is preferred. Another innovative approach is through full hydrogenation followed by IE with the native oil. In all cases, IE results in a myriad of TAGs and these raise questioned about their nutritional adequacy. The bulk of published evidence suggests IE fats are not significantly different from their native fats for plasma lipid responses in the fasted state. However, in the postprandial state differences in plasma TAG clearance may result due to IE mediated alterations in the sn1, 3 and sn2 stereospecificity within the TAG molecule. One study reported adverse outcomes on fasting plasma glucose and insulin resulting from a diet predominated by IE stearic rich fat. A follow up human study has examined impact of fats rich in palmitic or stearic versus their IE analogues. Preliminary data suggest IE palmitic-rich diet significantly increased total, LDL-C and HDL-C compared to unmodified palmitic or stearic-rich fats (both IE and native); resulting TC/HDL-C ratio was not significantly different between diets. Plasma glucose was however significantly increased by the IE stearic-rich fat compared to all other treatments. These and other published evidence suggest that nutritional outcomes of IE fats are modulated by both fatty acid composition and/or their placements on the sn positions within the TAG molecules. Overall more research is advocated before results are translated into food legislations or practices.

Novel biosynthetic pathway from alpha linolenic acid to EPA through non-methylene interrupted fatty acid in HepG2 cell

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Background: n-3 Polyunsaturated fatty acid (n-3PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are converted from alpha linoleic acid (ALA) in mammalian tissues and cells. Generally, it is understood that the majority pathway of n-3PUFA biosynthesis is started from delta6-desaturation of ALA. On the other hand, it is also reported that ALA is converted into 20:3n-3 by elongation. These reports suggested that there are multi pathways for n-3PUFA biosynthesis.

Objective: We investigated the conversion of ALA in human hepatoma HepG2 cells and determined n-3PUFA biosynthetic pathway by RNA interference method.

Procedure: HepG2 cells were cultured with 50 microM ALA for 120 h. After incubation, the cells were washed by PBS and extracted total lipid for analysis of fatty acid composition by GC and GC-MS. Fatty acid composition of ALA-treated HepG2 cells which were silenced FADS1 gene were also analyzed for determination of n-3PUFA biosynthetic pathway.

Results: Three fatty acids converted from ALA were detected in HepG2 cells treated with 50 mM ALA and were identified 20:3n-3, 20:4n-3,6,9,15 (5c-20:4) and 20:4n-3,6,9,12 (8c-20:4) by GC-MS. Since 5c-20:4 content was decreased in HepG2 cells by silencing FADS1 gene, 5c-20:4 was converted via 20:3 from ALA by elongation and delta5-desaturation in HepG2 cells. In addition, the amount of EPA increased in HepG2 cells treated with 5c-20:4.

Conclusion: From these data, it is suggested that 5c-20:4 is converted from 20:3 by delta5-desaturation, and then to EPA by delta8-desaturation in novel biosynthetic pathway through non-methylene interrupted fatty acid. The present results indicate that the conversion from ALA into EPA in HepG2 cells depends on multi pathways.

Docosahexaenoic acid alters EGFR localization and inhibits signal transduction

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The epidermal growth factor receptor (EGFR) is integral in regulating cell growth and survival, making it an important target for colon cancer prevention. EGFR signaling is partially controlled by its localization to specialized, highly ordered, nanoscale regions of the plasma membrane, known as lipid rafts. Docosahexaenoic acid (DHA), a long-chain n-3 polyunsaturated fatty acid (PUFA), is known to perturb membrane domain organization through changes in lipid rafts. Therefore, we investigated the mechanistic link between EGFR function and docosahexaenoic acid (DHA) incorporation into the plasma

membrane. Both immortalized YAMC colonocytes (cell culture model) and C57BL/6 mouse colonic mucosa (in vivo model) treated with DHA exhibited a significant increase in EGFR phosphorylation accompanied by a paradoxical suppression of activation of ERK1/2, STAT3, and mTORC1, important downstream mediators of EGFR signaling. DHA also suppressed cell proliferation in an EGFR-dependent manner. The inhibitory effect on EGFR was specific to DHA, with other long-chain PUFA exhibiting no effect. We assessed each step in the EGFR-Ras signaling cascade to identify the locus of the DHA-induced disruption in EGFR signal transduction. Using total internal reflective fluorescence (TIRF) microscopy, we found that recruitment of Grb2 to EGFR was increased by DHA treatment. A pull-down assay for activated Ras indicated that Ras GTP binding, a lipid raft-dependent process, was significantly suppressed by DHA treatment. DHA treatment further antagonized EGFR signaling capacity by increasing EGFR internalization and degradation. Complementary colocalization and membrane subfractionation experiments demonstrated that DHA treatment reduced the segregation of EGFR into lipid rafts. From these results, we conclude that DHA elicits a novel suppression of EGFR signal transduction by altering the localization of the receptor within the plasma membrane. These findings have implications for understanding the molecular basis of dietary chemoprevention and other cellular processes in which DHA plays a central role.

Fatty Acid Metabolism in Cystic Fibrosis

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The fatty acid alterations are well-described in humans with cystic fibrosis (CF). One of the most consistent and earliest fatty acid abnormality is decreased linoleic acid (LA, 18:2ω6) in plasma and tissues that express cystic fibrosis transmembrane conductance regulator (CFTR). Other fatty acid abnormalities include low plasma docosahexaenoic acid (DHA, 22:6 ω3) and high eicosatrienoic acid (20:3 ω-9). Arachidonic acid (AA, 20:4 ω6) is usually not elevated in the plasma, but in cells and tissues. Possible mechanisms for decreased LA in CF are the activation of desaturases and elongases in the fatty acid biosynthesis pathway that convert LA to AA or increased release of arachidonic acid by activation of phospholipase A2 (PLA2). Increased AA may then play a role in the excessive inflammatory response of CF. Similar findings have been reported in human CF cells, CF mice and cell culture models of CF. To determine whether the plasma and tissue fatty acid profile of phospholipids was altered in CF pigs as in humans with CF, the fatty acid analyses were performed in plasma and liver of CF (CFTR−/−, n=18) and non-CF (CFTR+/− and CFTR+/+, n=13) pigs at birth. The plasma LA was significantly decreased whereas eicosatrienoic acid was significantly increased and AA was unchanged in the newborn CF pigs compared to non-CF. The plasma DHA was normal in CF pigs. The hepatic LA was significantly lower and eicosatrienoic acid and AA were significantly higher in CF pigs, compared to non-CF. There was no difference in the total fatty acid content of hepatic phosphatidylcholine, phosphaditylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, diphosphatidyl glycerol and sphingomyelin. However, the LA content was significantly lower and AA content was significantly higher in hepatic phosphatidylcholine and phosphaditylethanolamine. These results suggest that the fatty acid composition of phospholipds is altered in CF pig plasma and tissues and these changes can play a role in the disease pathogenesis.

Eicosapentaenoic acid in the treatment of skeletal muscle wasting in cancer cachexia

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Background: Cancer cachexia is a profound wasting condition affecting approximately 50% of cancer patients, for which there is no effective treatment. Skeletal muscle atrophy is a consequence of cachexia, however the mechanisms of wasting are yet to be elucidated. Systemic inflammation and oxidative stress are thought to play key roles in disease progression.

Objective: This study used a mouse model of cancer cachexia to investigate Eicosapentaenoic acid (EPA; Anti-inflammatory/antioxidant agonist) as a treatment to attenuate muscle wasting in cancer-associated cachexia. Female nude mice were inoculated with a cancer cachexia inducing cell line, then randomised into 2 groups – EPA treatment (0.4 g/kg), or no treatment (control). Animals were euthanised

29 days post-inoculation and muscle tissues harvested for analysis. Gene expression and enzyme activity analyses were carried out on gastrocnemius muscle.

Outcomes: The control group showed significant weight loss compared to initial weight from day 17 (P<0.01). EPA group showed no significant weight loss compared to starting weight, and weight was significantly higher, compared to the control group, for a total of 15 days. There was no significant change in gene expression of antioxidant components EcSOD, MnSOD, CuZnSOD, CAT, and NOX2 compared to controls. GPx expression was increased in the EPA treatment group (P<0.05). There was no significant change in activity of SOD, CAT, or GPx, and a significant increase in XO activity compared to controls (P<0.05).

Conclusion: Whilst EPA continues to show promise as a treatment in animal models, it does not appear that attenuation of weight decline is caused by increased antioxidant capacity. Increased XO activity in EPA treated mice indicates that it may potentially play a role reducing weight-loss in cancer cachexia, and warrants further investigation.

Cholesterol-induced domain formation and its implications for protein function.

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The thermodynamic properties of plasma membrane lipids play a vital role in many functions that initiate at the mammalian cell surface. Some functions are thought to occur, at least in part, because plasma membrane lipids have a tendency to separate into two distinct liquid phases, called liquid-ordered and liquid-disordered. We find that isolated cell plasma membranes are poised near a miscibility critical point separating these two liquid phases, and postulate that critical composition fluctuations provide the physical basis of functional membrane heterogeneity in intact cells commonly referred to as lipid rafts. In this talk I will describe several possible mechanisms through which dynamic fluctuations can be stabilized in super-critical membranes and will discuss implications for lipid dependent cellular functions. In addition, I will present some preliminary evidence suggesting that these structures can be visualized in intact cells using quantitative super-resolution fluorescence localization imaging..

Ruminant trans-11 vaccenic acid activates peroxisome proliferator-activated receptor-dependent pathways and improves cardiomyocyte hypertrophy associated with the metabolic syndrome

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Introduction: Trans-11 vaccenic acid (VA) and cis-9, trans-11 conjugated linoleic acid (CLA) and are natural trans fat present in ruminant-dairy foods. CLA has been shown to have numerous health benefits partially mediated by PPAR-dependent pathways. More recently, the independent lipid-lowering properties of VA have been reported in several animal models, but the underlying molecular mechanism remained unknown.

Objectives: (i) to assess the binding capacity of VA to PPAR-alpha/gamma in-vitro, (ii) to determine the effect of dietary VA supplementation on intestinal and hepatic PPAR-alpha/gamma expression in an animal model of the metabolic syndrome (the JCR:LA-cp rat) and (iii) to evaluate the effect of VA on cardiomyocyte hypertrophy in-vitro, which has been shown to be suppressed by PPAR agonists.

Methods and Results: The IC50 of VA and other fatty acids to PPAR-alpha/gamma ligand binding domains were assessed using competitive binding assays. The resultant inhibition curves indicate that VA is a potent ligand to both nuclear receptors, with greater binding capacity to PPAR-alpha relative to fenofibric acid. In-vivo, the intestinal mRNA and protein expression of PPAR-gamma were increased

(p<0.01 and p<0.05, respectively) in hyperlipidemic rats fed 1.0% VA for 16 weeks relative to control. No change was observed in the hepatic mRNA expression of PPAR-alpha or gamma between VA-treated and control hyperlipidemic rats. In addition, VA at concentrations of 30 and 100 μmol/L effectively suppressed endothelin-1 induced cardiomyocyte hypertrophy (p<0.01). Such inhibitory effect of VA was abolished with the presence of a specific PPAR-gamma antagonist.

Conclusion: Improvement in lipid metabolism and inhibition of cardiomyocyte hypertrophy as a result of VA supplementation may be partially accredited to the activation of PPAR-dependent pathways. These findings may thus provide impetus for further investigation of its clinical implications under dyslipidemic conditions, and for national and international food labeling regulations to differentiate VA from industrially produced trans fats.

Dietary N-6 PUFAs and their Influence on Tissue Arachidonic Acid Content

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Enrichment of tissue phospholipids with arachidonic acid is of concern because of its ability to be metabolized to bioactive eicosanoids known to contribute to chronic diseases such as cardiovascular disease, cancer and inflammatory disorders. The literature clearly demonstrates that dietary omega-3 (n-3) polyunsaturated fatty acids (PUFAs) in the context of a Western diet can lower tissue arachidonic acid content by as much as 30% with long chain n-3 PUFAs being much more effective that alpha-linolenic acid. However, the impact of dietary n-6 PUFAs are less definitive in the context of a Western diet. A systematic review of adult human trials regarding n-6 PUFA consumption suggests that modulation of arachidonic acid levels in serum/plasma and erythrocyte fatty acids by dietary n-6 PUFAs are influenced by the number of double bonds in the fatty acid; where arachidonic acid with 4 double bonds is more effective than gamma-linolenic acid with 3 double bonds, which is more effective than linoleic acid with 2 double bonds. In fact, increasing or decreasing dietary linoleic acid levels were not significantly correlated with changes in arachidonic acid levels in the phospholipid pool of plasma/serum or erythrocytes. Follow up studies with dietary linoleic acid and arachidonic acid in mice provided Western-like background diet recapitulates the human data. Our results suggest that consuming n-6 PUFA with 3 or more double bonds positively influence arachidonic acid content in the phospholipid pool of plasma/serum and erythrocytes, with null effects from linoleic acid in adults consuming Western-type diets.

Poster Presenters

Poster Sessions

Arachidonate content of membrane lipids determined more by balance of dietary PUFA than by amount of PUFA in diet

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Rats were fed twelve identical moderate-fat (25%-energy) diets, differing only in fatty acid profile, for 8 weeks (equivalent to ~1 y in humans) and fatty acid composition of heart, muscle, liver, brain, erythrocyte and plasma phospholipid measured. Diet SFA content ranged 8-88%; MUFA content 6-65%; n-6 PUFA content 3-70%; n-3 PUFA content 1-70% and diet PUFA balance (= n-3 as % total PUFA) ranged 1-86%. To mimic the normal situation, diet PUFAs were 18-carbon fats. Of all membrane fatty acids, arachidonic acid (20:4n-6) showed the greatest response to diet. Difference between maximum and minimum 20:4n-6 membrane content was 21%, 18%, 14%, 14%, 11%, & 2% for plasma, liver, erythrocyte, muscle, heart and brain respectively. For all tissues, diet PUFA balance was a much better predictor of 20:4n-6 content than was diet content of either n-6 or n-3 PUFA. Diet PUFA balance accounted for an average 91% of the variation in membrane 20:4n-6 content for the five tissues (and 82% for plasma phospholipids). Diet n-6 PUFA content could explain an average 36% of the variation in tissue 20:4n-6 content (and 49% for plasma). Diet n-3 PUFA content explained 58% and unexpectedly was a better predictor of membrane 20:4n-6 content than was diet n-6 PUFA content. From a lipidomic analysis of muscle phospholipids we show that this strong influence of diet PUFA balance is observed for all phospholipid classes, and from analysis of earlier studies by Mohhauer & Holman we show it is likely independent of total diet fat content. In view of the essential role of membrane 20:4n-6 as the source for important chemical messengers such as eicosanoids, prostacyclins, endocannabinoids etc., if this finding in rats also applies to humans, it has very significant implications for the role of diet PUFA balance in combating a number of human diseases.

Application of an improved high-throughput lipid extraction method for the analysis of changes in brain lipid species in Parkinson's disease

Abbott, Sarah K.; Halliday, G.M.; Mitchell, T.W.; Brown, S.H.J.; Garner, B.

We have developed a lipid extraction protocol suitable for high-throughput lipidomic analysis of human brain samples. Protocol comparisons were made between the well-established Folch extraction (using glass-glass homogenisation) and a modified method which employed mechanical homogenisation (Bertin Technologies Precellys 24 mechanical homogeniser) and replaced chloroform with methyl-tert-butyl ether (MTBE). This improved method enabled us to significantly reduce sample handling time and increase efficiency compared to glass-glass homogenising. Furthermore, replacing chloroform with MTBE is safer (less carcinogenic/toxic) with lipids dissolving in the upper phase, allowing for easier pipetting and the potential for automation (i.e. robotics). Both methods were applied to post-mortem human brain tissue from Parkinson's disease (PD) patients (n=9) and age-matched controls (n=10) (obtained from the NSW Brain Banks following institutional approvals). Lipid species (including ceramide, sphingomyelin and glycerophospholipids) were analysed via electrospray ionisation mass spectrometry (ESI-MS) using an AB SCIEX QTRAP® 5500 and quantified using appropriate internal standards. No differences in lipid species composition were evident between the lipid extraction protocols. Significant changes in sphingolipid composition were observed in PD subjects when compared to control subjects, particularly in regions known to accumulate Lewy body-related pathologies. The concentration of most sphingomyelin and ceramide species (nmol/g tissue) was significantly decreased in PD cases. Fatty acyl chain length of ceramide and sphingomyelin species was also altered in PD, with a significant increase in species with shorter chain length (≤20-carbon) and corresponding decrease in species with longer chain length (>20-carbon). Reductions in sphingolipid levels and changes in fatty acyl chain length may be related to important alterations in sphingolipid metabolism, such as loss of functional glucocerebrosidase or reduced ceramide synthase expression.

Association Between the Changes in Body Fatness and Fatty Acid Composition of Plasma Phospholipids During the Early Pubertal Period

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Aim: Plasma fatty acid composition can change with age, reflecting not only diet but also levels of desaturating enzymes such as stearoyl-CoA desaturase (SCD), delta-6 desaturase (D6D) and delta-5 desaturase (D5D) that contribute to the development of insulin resistance. This study analyzed longitudinal changes in fatty acid composition in Japanese children during the early pubertal period and the association with the changes in desaturase indices on body fatness and insulin resistance.

Methods: The study included 77 children, 38 boys and 39 girls, aged 9.6 ± 0.5 years (mean $\pm$ SD). Relative weight (RW) and the waist-to-height ratio (WHtR) were determined, the fatty acid composition of plasma phospholipids was analyzed by gas chromatography, and the desaturase indices were calculated: SCD (16:1n-7/16:0: SCD16 and 18:1n-9/18:0: SCD18), D6D (20:3n-6/18:2n-6) and D5D (20:4n-6/20:3n-6) in 2006 and 2009.

Results: Longitudinal changes in fatty acid composition were generally similar in both sexes. However, an increased D6D index and a decreased D5D index were associated with a RW increase in boys, while significant relationships were demonstrated in both sexes for the WHtR. In addition, an increase in the D6D index was associated with an increased HOMA-R only in girls.

Discussion: The sexual dimorphism of the association between the desaturase indices and body fatness may be explained by sex-specific pubertal changes in body composition. The changes in the desaturase indices were also significantly associated with the changes in RW in boys but not in girls. Further studies should be done to investigate the interaction between desaturases and the changes in growth and sex hormones.

Conclusion: There were no sex-related longitudinal changes in fatty acid composition, but the association with the changes in body fatness and insulin resistance were sex-specific.

Water dispersible plant sterol shows improved lipid lowering efficacy compared to plant sterol ester

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Introduction: Dietary extrinsic sugars, chiefly sucrose and fructose, have been implicated in the formation of abnormalities in plasma lipoproteins associated with increased cardiovascular risk, primarily by elevating plasma triglyceride (TG). The extent to which these sugars can elevate plasma TG and alter lipoproteins may depend on their ability to promote the accumulation of fat and insulin resistance in the liver. Methods: The influence of liver fat on the plasma TG response to dietary sugars was examined in a dietary intervention study in which plasma TG was measured before and after two, 12 week diets that were high and low in extrinsic sugars. The diets were delivered in a randomised cross-over, with a 4 week wash-out, to male participants (n=25, aged 40-65 years) at increased cardio-metabolic risk as defined by published criteria. The diets exchanged two-thirds of dietary carbohydrate with foods high and low in extrinsic sugars, and achieved target ratios of starch to sugar of 1:1.2 (high sugar) and 3:1 (low sugar). Liver fat was measured at baseline by magnetic resonance spectroscopy (MRS). Results: there was a significant difference in plasma TG between the two diets (p=0.003). Plasma TG generally increased and decreased on the high and low sugar diets, respectively, across increasing quintiles of percentage liver fat. Subdivision of the cohort around the median of percentage liver fat (4.2%) revealed a significant difference in plasma TG between groups with moderately high (n=12) and low liver fat (n=13) after the high sugar diet (p=0.02), but not the low sugar diet. Conclusion: These data indicate that an elevated liver fat can influence the capacity of extrinsic sugars to increase plasma TG, and support the role of liver fat in mediating the potentially adverse effects of extrinsic sugars on plasma lipoproteins. Research sponsor: Biotechnology & Biological Sciences Research Council (Grant No. BB/G009899/1).

Characterization of novel synthetic fatty acid analogues in HepG2 cells

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Various long chain fatty acids impact cell function, viability and gene regulation. Palmitate (C16:0) is well tolerated at low micromolar concentrations but causes toxicity at higher concentrations. In contrast, the monounsaturated fatty acid oleate (C18:1) is well tolerated up to 1 mM. These properties may impact the establishment and progression of diseases such as type 2 diabetes, cardiovascular disease and some cancers in a process called lipotoxicity. In the present work we have characterized novel long chain fatty acids containing cyclobutene or cyclobutanone groups. In HepG2 cells the compounds did not inhibit uptake of the fluorescent fatty acid C1-BODIPY-C12. Effects on cellular viability as assessed using the MTT assay was variable. Uptake into cells and incorporation into complex lipids was verified using HP-TLC and GS/MS demonstrating that they can substitute for natural fatty acids. Currently, we are evaluating whether or not the compounds alter gene expression using the fatty acid synthase, Scd1, EHHAD and CD36 genes as targets because their expression is known to be altered by exogenous fatty acids. These compounds offer the opportunity to further explore the impact of fatty acid chain modifications on fatty acid uptake, metabolism and fatty acid-dependent gene expression.

Water dispersible plant sterol shows improved lipid lowering efficacy compared to plant sterol ester

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Despite repeated demonstration of efficacy of plant sterols (PS) as cholesterol-lowering agents in humans, issues surrounding reduced PS bioavailability in some dietary formulations remain to be elucidated. The aim of this clinical study was to determine the efficacy on plasma cholesterol-lowering of a water dispersible formulation of plant sterol (WD-PS) preparation versus plant sterol esters (PS-ester), consumed within dairy products. Forty-seven hyperlipidemic subjects (25 males and 22 females, age 19-75 years at baseline) completed the double-blind, randomized, crossover study. Subjects consumed a single-dose daily, for 4 wk, each of the following 3 treatments: (i) a yogurt only (control) or the same yogurt supplemented with 2 g PS equivalents provided as either (ii) WD-PS or (iii) PS-ester. A free living study was conducted without controlling diet during the 4 wk supplementation period. Yogurts enriched with WD-PS or PS-ester induced similar decreases in serum total cholesterol (7.7% and 6.3%, respectively) and LDL cholesterol levels (11.7% and 11.6%, respectively), as percentage relative to the control group. The ratios of total cholesterol to HDL cholesterol and non-HDL cholesterol to HDL cholesterol were significantly decreased ($p \leq 0.05$) with WD-PS (10.6% and 15.2%) but not with PS-ester (7.0% and 10.8%), respectively. Over the treatment period, consumption of WD-PS significantly reduced serum triglycerides (13.9%) as compared to consumption of PS-esters. Moreover, plasma HDL-C levels demonstrated a trend ($p=0.22$) towards increasing concentration levels (2.7%) in the WD-PS phase, compared to control, while, there was a decline in HDL cholesterol levels after consumption of PS-ester (-0.04%). Both plant sterols, WD-PS and PS-ester, contributed effectively to LDL cholesterol lowering. However, the new formulation of WD-PS appeared to yield additive lipid lowering effects by improving serum TG and the ratio of total cholesterol to HDL cholesterol compared to PS-ester.

Interactions Between Membrane Fatty Acid Transporter CD36 and Ca2+ Signaling in Cardiomyocytes

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Background: CD36 is a multifunctional membrane protein that occurs in different cell types and interacts with diverse ligands. In cardiomyocytes, CD36 functions as a long-chain fatty acid transporter to facilitate substrate uptake. In taste bud cells, CD36 acts as a lipid sensor, and was shown to trigger recruitment of intracellular Ca2+ from endoplasmic reticulum upon linoleic acid exposure. Whether CD36 also affects Ca2+ signaling pathways in cardiomyocytes has not been studied yet.

Objective: To explore whether CD36 has a role as a signal transducer in cardiomyocytes by triggering Ca2+ signaling and study the possible consequences in terms of modulation of Ca2+ channels in healthy and diabetic rodent heart.

Procedure: Cardiomyocytes from CD36-deficient (CD36-/-) mice and control (wild type C57Bl/6) mice were treated with palmitate, sulfo-N-succinimidyl oleate (SSO, cell-impermeable CD36 inhibitor), Ca2+ ionophore (A233187), or thapsigargin (inhibitor of intracellular SERCA type Ca2+ pumps). Subsequently, intracellular Ca2+ fluxes of single cells (Fluo-4-AM loaded) were measured by fluorescence microscopy in real-time.

Results: Cardiomyocytes from CD36-deficient mice show elevated levels (20¬¬–30%) of basal intracellular free Ca2+ compared to cells from wild type mice. Palmitate (180uM), Ca2+ ionophore A23187 (5uM) and SSO (500uM) treatment induced a 52%, 44% and 54% further increase, respectively, in intracellular free Ca2+ fluxes in cardiomyocytes from CD36-deficient mice compared to wild type mice.

Conclusion: Our results show that the presence of CD36 prevents elevated intracellular free Ca2+ fluxes. We suggest that CD36 has a role in the modulation of intracellular Ca2+ levels perhaps by acting on Ca2+ channels. Results of the study are novel and needs further experiments at a mechanical level.

Dietary Fish Oil Delays Neutrophil Recruitment Early During Endotoxin-Induced Inflammation but Enhances Their Recruitment Later In the Inflammation

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Background: Omega-3 polyunsaturated fatty acids affect neutrophil recruitment in vitro and may have beneficial effects in inflammation where neutrophil migration and activation is of importance.

Objective: To determine the effects of dietary fish oil on neutrophil numbers and subpopulations in healthy and inflamed mice.

Procedure: Mice were fed a Western-type diet without (C) or with 2.8% fish oil (FO) for 6 weeks. They were injected intraperitoneally with lipopolysaccharide or not and blood and peritoneal fluid collected at various time points. Cell numbers, size, granularity and expression of surface molecules and chemokine receptors was analyzed with flow cytometry.

Results: Dietary fish oil did not affect the number of neutrophils in blood or peritoneum of healthy mice. In inflamed mice fed fish oil there was a tendency towards more neutrophils in blood 12 and 24 h following endotoxin administration, but fewer peritoneal neutrophils at 12 h and a trend towards fewer at 24 h than in inflamed mice fed the control diet. However, there were more neutrophils in the peritoneum of mice in the FO group 48 h after LPS administration than in mice in the C group. Inflamed mice had two neutrophil populations in the circulation; the one present in healthy mice (N1) and another with larger, less granular neutrophils expressing more CD11b and Ly6G (N2). Inflamed mice in the FO group had a higher proportion of N2 neutrophils in the circulation.

Conclusion: These results indicate that although dietary fish oil may delay the recruitment of neutrophils from blood to the peritoneum early in inflammation it can increase the number of peritoneal neutrophils at later time points. This may be of benefit later in the immune response as impaired neutrophil migration

and activation has been linked with the immunosuppression that occurs in the later phases during the inflammatory process.

Complete fatty acid profiling using a single LC-MS based lipidomic approach

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Fatty acids are present in biological samples both in unesterified (free) and esterified forms. Their analysis usually requires multi-step procedures, which includes enzymatic or chemical hydrolysis and subsequent derivatization. These laborious procedures not only affect the sensitivity of detection, but also lead to a loss of information regarding the actual fatty acyl composition of different complex lipid classes (e.g., glycerolipids, glycerophospholipids, sterol lipids and sphingolipids). Here we present a robust method for the simultaneous profiling of free and esterified fatty acids from a variety of biological samples using ultra performance liquid chromatography (UPLC) coupled with ion mobility-mass spectrometry (HDMS). To demonstrate the potential of our approach, we used total lipid extracts from mammalian heart, liver, brain and plasma. The rapid separation of UPLC coupled with a post ionization separation by ion mobility substantially increased the peak capacity and therefore the number of lipids detected. By alternating low and elevated collision energy, we were able to simultaneously collect data on the free fatty acids and fatty acids esterified to complex lipids . Fatty acyl groups information was derived by either neutral loss in positive mode or formation of charged fragment ions in negative mode. Post-acquisition analysis differentiated the fatty acyl content and distribution in various lipid classes, according to the different biological samples analyzed. Fatty acyl composition could be visualized as 3D maps, which translated into molecular fingerprints of the various tissues analyzed. This method allows the comprehensive screening and fingerprinting of fatty acid composition, which holds promise for phenotype identification and comparative lipidomic analysis.

Conjugated linoleic acid as dietary therapeutic strategy for the treatment of neurodegenerative disorders

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Neurodegenerative disorders are often associated to lipid metabolic alterations and secondary inflammatory processes, which can contribute to the progression of the diseases. Lipid and steroid hormone pathways are altered, as an example, in X-linked Adrenoleukodystrophy (X-ALD), a demyelinating disorder characterized by the abnormal accumulation of very long chain fatty acids (VLCFA). A mixture of glyceryl trioleate and glyceryl trierucate (GTOE), well known as Lorenzo's Oil, is able to normalize lipid metabolic alterations in patients although not always the progression of the disease. Our objective was to introduce conjugated linoleic acid isomers (CLA) as therapeutic approach for X-ALD and for other neurodegenerative disorders.

In a first study we tested a mixture of GTOE (40 g/day) with CLA (5 g/day) for 2 months, in female heterozygous X−ALD individuals, to determine whether CLA is detected in the liquor and exerts a synergistic effect with GTOE. Since secondary inflammatory processes are present in this pathology, we evaluated Somatosensory evoked potentials (SEPs), interleukins, and other standard biochemical and clinical parameters.

After the treatment, CLA was detected in the liquor indicating the passage through the blood-brain barrier and the mixture decreased the VLCFA 26:0 and the ratio 26:0/22:0 in plasma. SEPs were improved after the treatment with the mixture, whereas with dietary GTOE were found unchanged. Inflammation is known to be a crucial factor for X-ALD pathogenesis and other disorders. This concerted action results in an improvement of the SEPs, which is a sign of neurological improvement.

Clinical studies are ongoing for testing GTOE+CLA in twelve males ALD patients with different phenotypes. Similar results as for female carriers were obtained. Our results are opening the field for a novel promising therapeutic strategy for X-ALD and other neurodegenerative disorders where inflammation plays a central role.

Dietary triacylglycerols with Palmitic Acid in the 2-Position modifies endocannabinoid and congener profile in rat visceral adipose tissue

Banni, Sebastiano; Gianfranca Carta, Elisabetta Murru, Claudia Vacca, Annarita Sirigu, Antonio Piras, Maria Collu, Hiskias Keizer, Luisa Gambelli, Alfred Haandrikman

Several lines of evidence suggest that the position of palmitic acid in dietary triacylglycerols (TAG) influences different biological functions. Human breast milk contains highly structured fats with >75% of palmitic acid at sn2 of the TAG backbone (2-PATG). A major incorporation of palmitic acid and/or a perturbation of fatty acid incorporation into membrane phospholipids (PL) may have an impact in the biosynthesis of bioactive lipids such as endocannabinoids and congeners which in turn may influence body composition homeostasis by modifying food intake, energy expediture and body fat distribution.

In order to verify whether 2-PATG increases palmitic acid incorporation into PL and thereby modifies fatty acid profile and biosynthesis of endocannabinoids and their congeners, we fed rats for 5 weeks a diet containing high concentration of 2-PATG and a diet contening low concentration of 2-PATG . Both diets had the same level of palmitic acid.

Rats on a diet rich in 2-PATG had a higher feed efficiency wich was associated to a decrease of the endocannabinoid anandamide (AEA) and concomitant increase of its congener palmitoylethanolamide (PEA) in visceral adipose tissue. Changes in the content of AEA and PEA may be explained by the increased incorporation of PEA precursor palmitic acid into phospholipids. We may conclude that dietary palmitic acid in sn-2 of the TAG backbone favors incorporation of PA into adipose tissue PL, perturbing endocannabinoid and congeners biosynthesis. Decrease of AEA and increase of PEA may elicit the increased feed efficiency and may also favour a decrease of visceral adipose tissue and thereby ectopic fat accumulation.

Therefore, 2-PATG may be suitable for dietary strategies aimed at improving feed efficiency and a more physiological fat distribution in the subcutaneous adipose tissue rather than visceral adipose tissue thus improving insulin sensitivity and preventing ectopic fat accumulation.

High Levels of Long Chain Polyunsaturated Fatty Acids in Cord Serum Predict Allergy Development in Childhood

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Background: Allergies increased strongly during the 20th century. The cause is unknown, but reduced stimulation by microbes in early childhood, as well as changed dietary habits may play a role. The consumption of long-chain poly-unsaturated fatty acids (LCPUFAs) has increased in the last decades.

Objective: To investigate whether cord serum levels of LCPUFAs are associated with the risk of allergy development in childhood.

Methods: Children were selected from a population-based birth-cohort comprising children born in Northern Sweden in 1996-7 who answered a questionnaire and underwent skin prick test at 13 years of age in 2009-10. We selected adolescents with either atopic eczema (n=40) or respiratory allergy (n=48), as well as skin prick negative asymptomatic controls (n=48). Cord serum and maternal serum had been obtained at birth and stored frozen. The proportion of eight LCPUFAs of the n-3 and n-6 series, and one saturated fatty acid were retrospectively analyzed in infant cord serum and maternal serum.

Results: The higher the levels of n-3 LCPUFAs, n-6 LCPUFAs, and total LCPUFAs in cord serum, the higher risk to have developed respiratory allergy at 13 years of age, and the lesser risk of being healthy (ptrend<0.001 for each comparison). In contrast, controls had significantly higher levels of the saturated fatty acid 20:0 in cord serum than those who developed eczema (p<0.001) or respiratory allergy (p=0.001). There was no correlation between maternal levels of serum FA levels and the levels of the same FAs in cord serum.

Conclusion & Clinical Relevance: Exposure to high levels of LCPUFAs in early infancy may be a risk factor for allergy development, suggested by modulating the function of the infant's developing immune

system. Current recommendations of increased intake of PUFAs in pregnant women and children may be questioned with regard to the risk of allergy development.

Effects of n-3 fatty acid and iron depletion and repletion, alone and in combination, on brain monoamines and spatial working and reference memory in rats

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Background: Deficiencies of n-3 fatty acids (n-3 FAD) and iron (ID) may impair brain development and function through shared mechanisms. However, little is known about potential interactions between these two common deficiencies.

Objective: We studied the effects of n-3 FAD and ID, alone and in combination, and examined, in rats with concurrent n-3 FAD and ID, whether repletion with Fe and/or n-3 FA, corrects deficits associated with deficiency.

Procedure: Rats were fed an n-3 FAD, ID, n-3 FAD+ID or a control diet for five weeks post-weaning, after n-3 FAD had been induced over two generations. At PND 56, n-3 FAD+ID rats were repleted for five weeks with docosahexaenoic (DHA)/eicosapentaenoic acid (EPA) and iron, alone and in combination. Spatial working and reference memory (using the Morris water maze), brain monoamine metabolism, brain FA composition and iron concentrations were assessed after depletion and repletion.

Results: Dopamine (DA) and serotonin concentrations were additively increased and decreased, respectively in n-3 FAD+ID rats. n-3 FAD significantly increased norepinephrine concentrations 2 to 3-fold in olfactory bulb and frontal cortex (FC), but n-3 FAD+ID attenuated this increase. n-3 FAD and ID significantly impaired working memory performance and the impairment positively correlated with DA concentrations in FC. ID+n-3 FAD synergistically impaired reference memory performance.

Repletion with DHA/EPA or Fe alone reduced DA concentrations in FC, and DHA/EPA+Fe resulted in a greater reduction. DHA/EPA+Fe did not significantly improve working memory performance, and repletion with DHA/EPA alone exacerbated deficits. In the reference memory task, Fe+DHA/EPA improved learning behavior, but Fe or DHA/EPA alone did not.

Conclusions: Combined deficiencies of n-3 FA and iron disrupt brain monoamine metabolism and produce greater deficits in reference memory than ID or n-3 FAD alone. Furthermore, provision of either Fe or DHA/EPA alone in rats with both ID and n-3 FAD exacerbates the cognitive deficits associated with the combined deficiency.

Thirst deficits in aging are reversed by dietary omega-3 fatty acids or cyclooxygenase inhibition

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During heat waves morbidity and mortality rates in the elderly are dramatically increased. The elevated mortality rates are often the result of a failure to maintain adequate hydration. Elderly animals and humans have a reduced sensation of thirst and fluid intake when challenged by stimuli that typically induce drinking in younger adults. With aging there are increased omega-6 fatty acid derived prostaglandins (PG) in the midbrain; these prostanoids are known to inhibit fluid intake following dehydration. Increasing tissue omega-3 fatty acids promotes a reduction in the production of omega-6 fatty acid derived PGs. Therefore, we examined the effect of dietary omega-3 fatty acid supplementation on thirst and fluid intake in aging. Young adult (2 month old) and aged (22 month old) male Brown Norway rats were maintained for 4 months on an omega-3 fatty acid deficient diet or omega-3 fatty acid supplemented diet. After 2 months on the diet animals were subjected to a battery of thirst stimuli including 24 hour water deprivation, acute thermal dehydration, injection of hypertonic saline and injection of angiotensin II. Consistent with previous reports, aged animals had an impaired fluid intake response compared with young adult animals following both dehydration stimuli and hypertontic saline injection. Fluid intake responses were restored by dietary omega-3 fatty acid supplementation to the level of young adult animals; dietary omega-3 fatty acids did not alter fluid intake responses in young adult animals. Omega-3 fatty acid supplementation reduced mRNA expression of genes related to prostaglandin synthesis and hypothalamic PGE2 levels but had no effect on urine output, plasma vasopressin or atrial

natriuretic peptide. A similar restoration of fluid intake responses was observed in aged animals treated with a cyclooxygenase inhibitor. Together these results provide the first evidence that prostanoids are involved in the diminished thirst and fluid intake responses in aged animals.

Retro-conversion of docosahexaenoic acid in Caco-2 cells

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Background: Long-chain polyunsaturated fatty acids are metabolized in mitochondria and in peroxisomes. In terms of catabolism, the conversion of highly unsaturated fatty acids into shorter or more saturated fatty acids is predominantly localized in hepatocytes. However, a previous study suggests a possible involvement of human intestinal epithelium, following early detection in plasma of retro-converted products upon feeding with docosahexaenoic acid (DHA) [1].

Objective: Our aim was to highlight the ability of differentiated Caco-2 cells to retro-convert DHA.

Procedure: Differentiated Caco-2 cells are widely used as a validated model of the human absorptive enterocytes. Caco-2 cells were first cultivated for 14 days in DMEM containing 10% of FBS and then incubated for 7 days in the presence of DHA at concentrations ranging from 0 to 150 µM. No cytotoxicity was detected. Cells were then harvested, lipids extracted and fatty acid profiles determined by gas chromatography or liquid chromatography coupled to mass spectrometry.

Results: The addition of increasing concentrations of DHA in the culture medium of Caco-2 cells increased the accumulation of DHA by the cells. Concurrently, increasing amounts of eicosapentaenoic acid (EPA) appeared in cells. Furthermore, increasing amounts of C24:6 were obtained. In addition, the presence of C24:5 was observed.

Conclusion: For the first time in human intestinal epithelial cells, a retro-conversion of DHA into EPA was observed. The appearance of C24:6 and C24:5 suggests that the metabolic pathway already known for the conversion of EPA into DHA could also be used to retro-convert DHA into EPA.

1   Brossard N. et al. (1996) Retroconversion and metabolism of [13C]22:6 n-3 in humans and rats after intake of a single dose of [13C]22:6n-3 triacylgylcerols. Am. J. Clin. Nutr. 64: 577-586.

Disappearance of Long Chain Omega-3 Fatty Acids from Human Red Blood Cells (RBC) In Vivo after Supplementation with Salmon Oil

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BACKGROUND: Long chain Omega-3 Fatty Acids are essential dietary components indispensable for growth, development and general health. Little is however known about the kinetics of Omega-3 fatty acid metabolism. This probably explains the lack of a scientific basis for a universally accepted daily intake of long-chain Omega-3 fatty acids.

OBJECTIVE: To study the metabolism of long chain fatty acids in humans by following the disappearance of Omega-3 fatty acids from red blood cells in vivo after supplementation with salmon oil for six weeks.

METHODS: Eight healthy volunteers with normal blood lipid values were supplemented with capsules containing 1000 mg salmon oil (180 mg Eicosapentaenoic (EPA) and 120 mg Docosahexeanoic (DHA) acid) for six weeks. Average intake was 1000mg EPA and DHA per day. Blood samples were collected before supplementation, after six weeks of supplementation and during six weeks after cessation of supplementation. Lipids were extracted from washed RBC with chloroform-methanol and separated by thin layer chromatography. Methyl esters of fatty acids from the different lipid fractions were analyzed by gas liquid chromatography (GLC) and expressed as percentage of fatty acids.

RESULTS: The EPA and DHA content of Phosphatidyl Choline (PC) and Phosphatidyl Ethanol Amine (PEA) of red cells increased significantly during supplementation. After cessation of fish oil

supplementation, the rate of disappearance of EPA from RBC-PC was about 12 X faster than that measured for DHA. However similar rates of disappearance were observed for EPA and DHA in PEA.

CONCLUSION: Significant differences were observed between the rate of metabolism of EPA and DHA in red blood cells

The Effects of Dietary n-3 Polyunsaturated Fatty Acids on Fatty Acid Composition and Location of Proteins in Rat Heart Lipid Rafts

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Background: Location of proteins in lipid rafts of the cardiomyocyte cell membrane is important for transmembrane signaling. Studies have shown that n-3 long chain polyunsaturated fatty acids (LC-PUFA) alter lipid composition and location of proteins in lipid rafts in human breast cancer cells, mouse colon and T-cells, but their effects on lipid rafts in heart muscle have not been investigated. Objective: The aim of this project was to study the effects of dietary n-3 LC-PUFA on lipid composition and location of adrenergic receptors in lipid rafts from rat heart.

Methods: Lipid rafts were isolated on a sucrose gradient from hearts of adult rats that had been fed a western diet without (control) or with 2,8% fish oil. Proteins and the ganglioside GM1 were analyzed in all 12 fractions of the sucrose gradient with western blots and dot blots, respectively. Cholesterol was measured with a spectrophotometric assay kit. Phospholipids were isolated from lipid rafts and their fatty acid composition analyzed with gas chromatography.

Results: The lipid raft markers caveolin3, flotlillin1, cholesterol and GM1 were present in fractions 4-6 from the top of the sucrose gradient and were in similar amounts in these fractions from both dietary groups. The proportion of n-3 LC-PUFA were higher and n-6 LC-PUFA lower in phospholipids of lipid rafts from rats fed the fish oil diet than in the control rats. The alpha1 adrenoceptors were located mostly in lipid rafts, but the beta1 adrenoceptors were found in both lipid rafts and the soluble membrane. The amount of these receptors was similar in lipid rafts from rats in the two diet groups.

Conclusion: Dietary fish oil led to partial replacement of n-6 LC-PUFA by n-3 LC-PUFA in phospholipids of lipid rafts from rat heart but did not affect the location of cholesterol, GM1 or protein in the lipid rafts.

Inhibitory effect of fucoxanthin, brown seaweed carotenoid, on hepatic stearoyl-CoA desaturase 1 (SCD1) in mice

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Background: Fucoxanthin (Fx) is a xanthophyll, which is found in edible brown seaweeds such as Undaria pinnatifida. Our previous study showed that Fx suppresses body weight gain and ameliorates hyperglycemia in obese/diabetic KK-Ay mice. Furthermore, we observed that dietary Fx markedly decreased oleic acid in the fatty acid composition of the liver lipid. This result suggests that the alteration of oleic acid composition is dependent on the inhibition of stearoyl-CoA desaturase 1 (SCD1) activity in the liver of KK-Ay mice by Fx.

Objective: The aim of this study was to investigate the relationship between the inhibitory effect on hepatic SCD1 and anti-obesity effect of Fx in KK-Ay mice. Since hepatic SCD1 has been reported to be a target of leptin function, we further investigated the effect of Fx on hepatic SCD1 expression and anti-obesity effect in genetically obese ob/ob mice without leptin production.

Procedure: KK-Ay mice and ob/ob mice were fed 0.2% Fx diet for 4weeks. Total lipids of tissues were extracted by Folch method, and then fatty acid composition was analysed by GC. SCD1 mRNA and protein expression levels were estimated by PCR and Western blotting.

Results: In the liver of KK-Ay mice, dietary Fx decreased the ratio of oleic acid to stearic acid in the fatty acid composition. SCD1 mRNA and protein expression levels were also significantly decreased to 55% and 10% of control KK-Ay mice, respectively. In addition leptin levels were significantly lowered in the Fx

group. These results indicate that dietary Fx improved leptin sensitivity in hyperleptinemia KK-Ay mice. However, these alterations and anti-obesity effect were not observed in leptin deficient ob/ob mice.

Conclusion: The present results suggest that dietary Fx exerts anti-obesity effect in KK-Ay mice through down-regulation of hepatic SCD1 expression in leptin dependent signaling pathway.

Differential effects of krill oil and fish oil on the hepatic transcriptome in mice

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Dietary supplementation with n-3 polyunsaturated fatty acids (n-3 PUFAs), specifically the fatty acids docosahexaenoic acid (DHA; 22:6 n-3) and eicosapentaenoic acid (EPA; 20:5 n-3), is known to have beneficial health effects including improvements in glucose and lipid homeostasis and modulation of inflammation. To evaluate the efficacy of two different sources of n-3 PUFAs, we performed gene expression profiling in the liver of mice fed diets supplemented with either fish oil or krill oil. We found that n-3 PUFA supplements derived from a phospholipid krill fraction (krill oil) downregulated the activity of pathways involved in hepatic glucose production as well as lipid and cholesterol synthesis. The data also suggested that krill oil-supplementation increases the activity of the mitochondrial respiratory chain. Surprisingly, an equimolar dose of EPA and DHA derived from fish oil modulated fewer pathways than a krill oil-supplemented diet and did not modulate key metabolic pathways regulated by krill oil, including glucose metabolism, lipid metabolism and the mitochondrial respiratory chain. Moreover, fish oil upregulated the cholesterol synthesis pathway, which was the opposite effect of krill supplementation. Neither diet elicited changes in plasma levels of lipids, glucose or insulin, probably because the mice used in this study were young and were fed a low fat diet. Further studies of krill oil supplementation using animal models of metabolic disorders and/or diets with a higher level of fat may be required to observe these effects.

Biological properties of sciadonic (5,11,14 20:3) and juniperonic (5,11,14,17 20:4) fatty acids

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Commercially-available vegetable oil-derived methylene-interrupted fatty acids are found in angiosperm plants. Non-methylene interrupted fatty acids (NMIFA) such as sciadonic (SCI; 5,11,14 20:3) and juniperonic (JUN; 5,11,14,17 20:4) are common in gymnosperm plant species, formed by Δ5-desaturation of 11,14 20:2 and 11,14,17 20:3, respectively. These NMIFA are rarely studied, but based on studies to date, have important anti-inflammatory properties. Herein, a systemic review of these fatty acids will be provided. SCI and JUN have structural similarity to ARA and EPA, respectively, but without the internal Δ8 double bond, are not converted to ARA and EPA. In mouse tissues and HepG2 cells, SCI is incorporated into PC and PE pools resulting in reduced levels of ARA. However, SCI has more selectivity for the PI and PIP2 pools, which could affect DAG-PKC signaling; and MAG signaling via interactions with CB receptors. SCI has poor affinity for PLA2, leading to substantial accumulation in PL pools when fed to mice. SCI and JUN are not major substrates for eicosanoid production. SCI suppressed production of pathologic anti-erythrocyte- and anti-double stranded DNA antibodies, and prolonged survival in autoimmune mice. In mice injected with collagen-adjuvant emulsions, mortality was lowest in mice fed Juniper oil with 11% SCI as compared to fish oil and controls. In mice fed Juniper oil and injected with LPS, PGE2, TXB2, 6-ketoPGF1α, IL-6, and IL-10 were decreased vs. controls, and Juniper oil was as effective as fish oil in decreasing these pro-inflammatory markers. Oils containing SCI can also lower plasma cholesterol in animal models. SCI is also incorporated into mouse skin phospholipids when applied topically, and reduced ARA, PGE2, and TPA-induced ear edema, suggesting benefits for skin inflammation. JUN has been very rarely studied, is not incorporated into PLs, and its biological properties are not known. The clinical utility of these NMIFA for treating inflammatory conditions, and next steps in understanding their biology will also be described.

Enteral DHA reduces the need of buprenorphine dose in surgical neonates at the neonatal intensive care unit

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Background: Most of the neonates born with cardiovascular malformations need thoracic surgery, and because chest is highly innervated, analgesic therapy is added to treatment. Buprenorphine, an opioid-derived analgesic, is used to ameliorate acute pain in surgical neonates. However, it produces several negative side effects such as respiratory depression, bradycardia, hypotension, and intestinal dismotility. Studies in animal models have shown that docosahexaenoic acid (DHA) has anti-nociceptive effects through the production of eicosanoids, resolvins, and B-endorphins, but similar analgesic effect in neonates has not been demonstrated.

Objective: To evaluate the effect of DHA supplementation on the buprenorphine requirement of neonates exposed to thoracic surgery during the neonatal intensive care unit stay.

Procedure: Secondary data analysis of a clinical trial which evaluated the effect of DHA on the inflammatory response of surgical neonates was performed. Thirty-six neonates programmed for cardiovascular surgery received daily 75 mg/kg DHA (G-DHA) (Neuromins for Kids, Martek Inc) or sunflower oil (G-SO) through enteral feeding from 2 days before to 6 days after the surgical procedure. The dose and length of use of buprenorphine were decided by attending doctors, blinded to the aim of the study, according to neonates clinical condition. Independent t-test and multiple linear regression models were carried-out.

Results: Eighteen neonates received sunflower oil and eighteen DHA. The buprenorphine accumulated dose (16.8±10.3 vs. 25.2±20.5 μg/kg, P=0.029) and the duration of use (2.7±1.7 vs. 4.2±2.2 d, P=0.030), were lower in the G-DHA than in the G-SO group. These results remained significant after adjusting by confounders such as human milk intake and the use of other drugs such as ketorolac and steroids.

Conclusion: These results demonstrate that DHA supplementation reduces the requirement of buprenorphine in neonates exposed to thoracic surgery.

Evidence of beneficial effects of enteral docosahexaenoic acid on cytokine production and clinical outcomes in surgical neonates

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Background: Surgical patients are on risk to build-up uncontrolled inflammatory response that predispose them to sepsis and multiorgan dysfunction. Since neonates have an immature immune system, they are in higher risk to develop adverse clinical outcomes (ACO).

Objective: To evaluate the effect of the acute, enteral administration of docosahexaenoic acid (DHA) to surgical neonates, on the cytokine production and ACO.

Procedure: Randomized double-blind design. Thirty three neonates programmed for cardiovascular surgery received 75 mg/kg/day of DHA, (G-DHA) (Neuromins-for-Kids, Martek Inc®), or sunflower oil (G-SO) by enteral feeding, two days before and throughout six days after surgery. Percentage of leucocytes producing intracytoplasmic cytokines IL-1beta, IL-6, TNF-alpha, IL-10 and IL-1ra in whole blood was determined by flow-cytometry at baseline, 24h and day seven post-surgery. ACO such as: presence of sepsis, respiratory, cardiovascular, hematologic, renal and hepatic dysfunctions, and length of hospitalization at neonatal intensive care unit were assessed.

Results: Fifteen neonates received DHA and eighteen SO. G-DHA showed lower percentage of IL1B+ cells at 24h (0.03±6.2% vs. 0.7±2.3%, P<0.045), TNF-alpha+ (3.5±2.2% vs. 6.8±3.3%, P<0.046) and IL-10+ (0.9±2.2% vs. 2.1±3.5%, P<0.025) than G-SO at 7-d post-surgery. Repeated-measures ANOVA to adjust by confounders, demonstrated that these percentages remained lower in G-DHA than in G-SO for IL-1beta+, IL-6+, IL-1ra+ and IL-10+, P<0.05. Likewise, G-DHA presented lesser frequency of ACO or

borderline lower than G-SO: sepsis (3 vs. 9, P=0.077); organ dysfunctions (17 vs. 2, P<0.001), but no difference in severe sepsis (1 vs. 4, P= 0.229). Logistic regression showed lower relative risk (RR) to develop one or more organ dysfunctions in G-DHA than G-SO (RR=0.031 [0.002, 0.528]. Length of hospitalization was shorter in G-DHA than in G-SO (6.9± 2.1d vs. 11.5±2.2d, P=0.035).

Conclusion: These results demonstrated that enteral DHA administration reduces the inflammatory response and improve clinical outcomes in neonates exposed to thoracic surgery.

Protective Effects of Vitamin E on Lipid Symmetry of Erythrocyte Membrane Against Ethion Induced Toxicity

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Ethion is a widely used organophosphate, which have been identified as contaminants. Present study was designed to ascertain the toxic effects of oral administration of ethion on erythrocytes and to see if the supplementation of vitamin E along with ethion could modulate these effects. Adult male albino (wistar strain) were orally administered ethion and vitamin E daily for 15 days. Animals were divided into four groups: control (corn oil only); ethion treated (2.7 mg/kg bw/day); vitamin E treated (100 mg/kg bw/day) and ethion and vitamin E treated. Erythrocytes and Erythrocyte membranes were prepared. There was a significant decrease in body weights of ethion intoxicated rats as compared to control and vitamin E treated rats. Lipid peroxidation increased significantly with the ethion exposure in the erythrocyte membrane. A significant decrease in total lipids, cholesterol, phospholipids and protein content of erythrocyte membrane was observed after ethion administration. A significant decrease in membrane bound enzymes such as total-ATPase, Mg2+ATPase, Na+K+ATPase, Ca2+ATPase and acetylcholinesterase was observed and as result there is increase in calcium uptake by ethion treated erythrocytes (as a function of time as well as concentration) as compared to controls. Scanning electron microscopy of ethion treated erythrocytes revealed that administration of ethion resulted in prominent morphological changes. It can be concluded from the present study that the ethion induced toxic effects on erythrocytes in terms of biochemical and morphological alterations and mechanism involved appears to be mediated through the increased lipid peroxidation, decreased membrane composition, decreased membrane bound enzyme activities lead to the impaired membrane functioning and ultimately resulting in altered morphology of erythrocytes. Alterations in calcium homeostasis was also observed in ethion treated erythrocytes. Vitamin E found to alleviate the toxic effects of ethion induced biochemical and morphological changes suggesting that vitamin E supplementation to individuals exposed to ethion as well as to other OP pesticides would be beneficial.

Stability of blood specimens in biobanks: Influence of storage conditions on fatty acid stability

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Fatty acid profiles in biological tissues are used as biomarkers of dietary exposure. Recently the omega-3 index (i.e. percentage EPA + DHA in red blood cells, RBC) has gained interest as biomarker for cardiovascular disease risk. The present research evaluate the stability of fatty acid profiles from different human blood sample specimens, specifically RBC, serum and plasma samples during a period of up to 52 weeks and in terms of temperature (-20 degrees C & -80 degrees C), antioxidant BHT (presence/absence) and thawing (single/multiple). In the present study, samples from one healthy woman were drawn and separate batches were stored for up to one year (and running). Replicates were analyzed with a fast methylation GC method generating 340 gas chromatographic fatty acid methyl ester profiles (8280 individual fatty acids) expressed as absolute (mg/g) and relative (%) concentrations. An excel macro with cut off for degradation and upconcentration sample ratios After/Fresh <0.7 and After/Fresh >2 respectively was used. The results indicate that RBC degrades faster at -20 degrees C than -80 degrees C. With BHT this effect is partially prevented. Plasma seems to be relatively stable at both temperatures, but more stable with BHT when thawed more than once. Serum on the contrary, was more unstable than plasma and RBC. BHT partially prevents this effect . Multiple thawing of RBC affected samples at -20 degrees C more than at -80 degrees C. Absolute levels of fatty acids reflected more the stability of fatty acids than relative levels, which compensate up to 100 %. Higher carbon number fatty

acids were generally more susceptible for changes. The present study suggests important implications when analysing fatty acids; like preanalytical considerations to be taken when drawing blood samples and careful judgments to be done when analyzing samples from old biobanks. Biological variation studies are warranted.

Docosahexaenoic Acid (DHA) and Arachidonic Acid (ARA) in Breast Milk of Brazilian Women in the First Six Months of Lactation

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Few studies have evaluated DHA and ARA levels in breast milk of Brazilian women. We collected milk samples from 232 women in Mutuipe and Laje, Bahia, Northeast Brazil one month (mean 35 days; range 26-58) and 6 months (mean 186 days; range 169-216) after term birth. Forty-two women had samples available for DHA and ARA analysis from both collections and 82 and 108 women had samples available only from 1 or 6 month collections, respectively. Fatty acids were analyzed by capillary gas chromatography. We performed descriptive statistics and the Kruskal-Wallis test. The Federal University of Bahia ethics committee approved the protocol. DHA and ARA levels (% total fatty acids) in milk from women with samples from 1 month only were significantly higher than those from women with samples from 6 months only: DHA median (interquartile range [IQR]) 0.18 (0.15-0.24) and 0.09 (0.08-0.16), 1 and 6 months, respectively, p<0.001; ARA median (IQR) 0.55 (0.48-0.60) and 0.51 (0.45-0.59), respectively, p=0.049. DHA and ARA levels from women with samples available from both 1 and 6 month collections (n=42) did not change: DHA median (IQR) 0.18 (0.15-0.24) and 0.18 (0.14-0.23), 1 and 6 months, respectively, p=0.392; ARA median (IQR) 0.52 (0.44-0.58) and 0.49 (0.45-0.56), respectively, p=0.712. Median (IQR) milk DHA and ARA levels for all samples combined (n=274) were 0.16 (0.10-0.22) and 0.52 (0.46-0.59), respectively.

Median values for DHA in breast milk from women from Northeast Brazil were lower than the average reported for worldwide breast milk (0.32%; Brenna et al 2007). ARA medians were slightly higher than the worldwide average of 0.47% (Brenna et al 2007). Strategies to help these women increase DHA intake during pregnancy and lactation could help increase breast milk DHA levels and ultimately benefit infants. (Sponsored by Mead Johnson Nutrition).

Effects of AMR101, a Pure EPA Omega-3 Fatty Acid, on the Fatty Acid Profile in Plasma and Red Blood Cells in Patients With Very High Triglycerides (Results From the MARINE Study)

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Purpose: AMR101 is an Omega-3 fatty acid (FA) agent containing ≥96% pure icosapent ethyl, the ethyl ester of EPA. Previous cardiovascular (CVD) outcomes studies correlate increased red blood cell (RBC) Omega-3 FAs with reduced CVD risk. This analysis from the randomized, double-blind, 12-week MARINE study evaluated the effects of AMR101 on the FA profile in plasma and RBCs and the relationship to triglyceride lowering.

Methods: Patients (N=229) with very high triglycerides (≥500 mg/dL) on stable diet with or without statin therapy were randomized to placebo, AMR101 2 g/d, or AMR101 4 g/d. Plasma concentration and RBC membrane content of 28 FAs was measured in 154 patients using a GC/FID method.

Results: Baseline mean plasma EPA levels were 41, 50, and 50 µg/g for placebo, AMR101 2 g/day, and 4 g/day, respectively; at study end, EPA levels were 37, 158, and 280 µg/g. AMR101 increased placebo-adjusted mean plasma EPA levels from baseline by 402% with AMR101 2 g/d (p<0.0001) and 792% (p<0.0001) with AMR101 4 g/d. The increase in EPA levels with increased AMR101 doses correlated with the TG-lowering effect. The mean placebo-adjusted arachidonic acid (AA)/EPA plasma ratio, a biomarker of inflammatory status related to CVD risk, was significantly decreased from baseline by 88% with AMR101 2 g/d (p<0.0001) and by 99% with AMR101 4 g/d (p<0.0001). Similar FA shifts were observed in RBCs. No statistically significant changes were seen with DHA, indicating that the TG-lowering effect of AMR101 is solely due to the increase in EPA. Levels of docosapentaenoic acid (DPA), a metabolite of

EPA, increased similarly. Overall, the fractional pool of Omega-3 FAs increased versus a decrease of the Omega-6 FAs.

Conclusions: AMR101 significantly increased EPA in plasma and RBCs, and caused other shifts in FA concentration and RBC membrane content that may be associated with reduced CVD risk.

Pharmacokinetics of Eicosapentaenoic Acid in Plasma and Red Blood Cells After Multiple Oral Dosing with AMR101 (Ethyl-Eicosapentaenoic Acid) in Healthy Subjects

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Objective: AMR101 is an omega-3 fatty acid agent containing ≥96% pure icosapent ethyl, the ethyl ester of eicosapentaenoic acid (EPA). In a randomized, open-label, multiple-dose, pharmacokinetic (PK) study, we characterized EPA PK in plasma and red blood cells (RBCs) at doses expected to significantly decrease triglycerides, and explored dosing regimens.

Methods: Healthy subjects (6 males, 6 females/group) were randomized to AMR101 for 28 days: Group 1: 2 g/day (one 1-g capsule BID); Group 2: 4 g/day (2x1-g capsules BID); Group 3: 2 g/day (2x1-g capsules QD); Group 4: 2 g/day (2x0.5-g capsules BID). EPA concentrations were measured in plasma (total and unesterified) and RBCs before morning dosing (Days 1, 14, 26, 28) and at serial times after the last Day 28 dose with liquid chromatography/mass spectrometry. Total EPA included all EPA forms: incorporated in phospholipids, triacylglycerols, cholesteryl esters, and unesterified EPA (free fatty acid). PK parameters were calculated with standard methods.

Results: Plasma total EPA increased from 19 μg/mL (mean baseline) to a peak (Cmax) of 366 μg/mL at 5 hours postdosing 4 g/day on Day 28. RBC mean EPA Cmax after 4 g/day was 89 μg/mL (baseline, 12 μg/mL). A small fraction (<0.5%) of plasma exposure to EPA was free acid. Study-wide, baseline-adjusted steady state means (SD) for half-life, clearance, and volume of distribution of total EPA were 79 (47) hours, 757 (283) mL/hr, and 82 (56) L.

Conclusions: EPA PK profile demonstrated a slowly cleared, extensively distributed molecule (as expected), and was similar between women and men. Steady state for total and unesterified EPA was reached by 28 days in plasma, while RBC levels were still slowly increasing. BID and QD regimens with the same daily dose were comparable for EPA area under the curve (AUC). AUC and Cmax after 4 g/day were ~double vs 2 g/day, indicating dose-linearity.

Δ4-Desaturation Coded By FADS1 Mediates The Last Step In Docosapentaenoic Acid (22:5n 6) Synthesis In Human Cells

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The fatty acid desaturase (FADS) genes code for the rate-limiting enzymes required for the biosynthesis of highly unsaturated fatty acids (HUFA). The accepted pathway for biosynthesis of the 4-5 unsaturated n-6 docosapentaenoic acid (4,7,10,13,16-22:5) requires microsomal elongation of adrenic acid (22:4n-6) followed by one round of peroxisomal chain shortening according to 22:4→24:4→24:5→22:5. Using a heterologous expression system in human cells, we show that 22:5n-6 is synthesized by direct Δ4-desaturation according to 22:4n 6→22:5n 6, independent of 24 carbon fatty acids. MCF-7 human breast cancer cells devoid of Δ6-desaturation activity were stably transfected with the coding region of FADS1 or empty vector controls. Cells transfected with FADS1 but not empty vector convert 22:4n-6 → 22:5n-6. High specificity mass spectrometry analysis reveals that 22:4n-6 is readily elongated to 24:4n-6 (22:4n 6 → 24:4n 6), but 24:5n 6, a required intermediate in the accepted pathway, is undetectable in both vector only and transfected cells. FADS1-transfected cells did not show activity toward 22:5n-3. FADS1 is well known to be the final, rapid step in arachidonic acid biosynthesis via Δ5-desaturation, 20:3n-6 → 20:4n-6, as well as eicosapentaenoic acid (20:5n-3) synthesis.   Observation of Δ4-desaturation activity is analogous to a recently described dual Δ4/Δ5-desaturation in the rabbitfish to synthesize docosahexaenoic acid (4,7,10,13,16,19-22:6). The relevance of FADS1 Δ4 desaturation activity to biosynthesis of C22 HUFA will be discussed.

A novel fatty acid desaturase 1 (FADS1) isoform potentiates FADS2-mediated production of eicosanoid precursor polyunsaturated fatty acids

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The fatty acid desaturase (FADS) genes code for the rate-limiting enzymes required for the biosynthesis of highly unsaturated fatty acids (HUFA). Here we report discovery and function of a novel FADS1 splice variant. Rapid amplification of cDNA ends (RACE) was used to detect splice variants of FADS1 gene using non-human primate liver tissue. A series of FADS1 isoforms generated by alternative splicing of exons, 5' and 3' untranslated region (UTR) heterogeneity were identified.   Six of the FADS1 transcripts showed variation only in the 3' UTR, a region putatively responsive to microRNA targeting. FADS1 alternative transcript 1 (AT1) had truncation of exon 1 and part of exon 2, and an unusually spliced 5' UTR. FADS1AT2 had truncated exons 6 and 12 and deletion of exons 7-11. Studies with stably and transiently transfected cells showed that the protein coding region of FADS1AT1 enhances desaturation mediated by FADS2, leading to increased production of arachidonic acid and eicosapentaenoic acid (EPA). To our knowledge, this is the first observation of the isoform of one gene modulating the enzymatic activity encoded by another gene. Multiple protein isoforms were detected by immunoblot in primate liver, thymus, and brain. In human neuronal cells their expression patterns are modulated by differentiation, and results in alteration of cellular fatty acids. FADS1, but not FADS1AT1, localizes to endoplasmic reticulum and mitochondria. Ribosomal footprinting demonstrates that FADS1, FADS2, and FADS3 genes are translated at similar levels. The non-catalytic regulation of FADS2 desaturation by FADS1AT1 is a novel, plausible mechanism by which several phylogenetically conserved FADS isoforms may regulate HUFA biosynthesis in a manner specific to tissue, organelle, and developmental stage.

Identification of a specific splicing regulator, polypyrimidine tract binding protein that modulates splicing of FADS2 alternative transcript 1

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The Δ6-desaturase/Δ8-desaturase, encoded by FADS2, plays a central role in highly unsaturated fatty acid (HUFA) synthesis. We have shown that all three FADS (FADS1, FADS2, FADS3) genes yield alternative transcripts (AT) via alternative splicing, UTR, and polyA site heterogeneity, and elsewhere at this meeting report that an alternative transcript of FADS1 enhances FADS2 function. There are no known factors that modulate FADS AT splicing. We report here an investigation of a known splicing regulator, the polypyrimidine tract binding protein (PTB, or hnRNP I), on pre-mRNA splicing of FADS2 to yield FADS2AT1. PTB is shown to bind an exonic splicing silencer element and repress alternative splicing of FADS2 into FADS2AT1. PTB and FADS2AT1 were inversely correlated in neonatal baboon tissues, implicating PTB as a major regulator of tissue-specific FADS2 splicing. In HepG2 cells, PTB knockdown modulated alternative splicing of FADS2, as well as FADS3, a putative desaturase of unknown function. Total omega-3 fatty acids decreased by nearly one half relative to omega-6 fatty acids in PTB knockdown cells compared to controls, with a particularly strong decrease in eicosapentaenoic acid (EPA) relative to arachidonic acid, representing a rare demonstration of a mechanism specifically altering the cellular omega-3 to omega-6 fatty acid ratio without any change in diet/media. These findings reveal a novel role for PTB and the first report of a factor regulating FADS alternative splicing.

Method Validation for the Measurement of Fecal Fatty Acid Soaps in Infants Fed Either Human Milk or Formulas Containing Unmodified Palm Oil

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Infant formulas may be formulated with vegetable oil blends to replicate the fatty acid profile of human milk. Although the palmitic acid content may be matched at approximately 20%, palmitic acid from vegetable oils is mostly at sn-1 and sn-3 positions whereas palmitic acid from human milk is mostly at the sn-2 or beta position (C-2 hydroxyl of the glycerol backbone) of the triglycerides. Since digestive

enzymes preferentially release fatty acids from the sn-1 and sn-3 positions, palmitic acid from vegetable oils may form mineral soaps with Ca2+ and Mg2+ which have been associated with constipation and reduced mineral and fat absorption.

We report a validation for measurement of palmitic acid soap for homogeneous QC pools of fecal samples from infants fed with either human milk (low QC) or infant formulas containing unmodified palm oil (high QC). Infant fecal samples were extracted to obtain the neutral lipids, including non-soap free fatty acids. The remaining sample was treated with acetic acid to release the soap fatty acids which were then isolated by a second reflux step. The free acids in each sample fraction were converted to methyl esters, then quantified using gas chromatography with flame ionization detection. The precision of the method (RSD) was <13% (n=6, one day) and <18% (n=18, three days). Recovery of palmitic acid or of palmitic acid soap, spiked as calcium palmitate from infant fecal samples was between 76 and 109%.

This method improves upon previous procedures with sequential rather than parallel extractions and lower sample size requirements. The validation data for homogeneous sample pools and also samples from a similar 3-year follow-up study clearly demonstrate its value. This precision and accuracy enabled the assessment and comparison of samples from a clinical intervention study designed to determine the efficacy of infant formulas with modified palm oil.

Antidepressant and cognitive effects of krill oil in rats

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Krill oil (KO) is an omega-3 supplement, where the majority of these essential fatty acids are bound to phospholipids. The research on the beneficial effects of omega-3 fatty acids in phospholipids is emerging with studies showing beneficial effects in models of obesity, inflammation and cardiovascular disease. The purpose of the present study was to evaluate the potential of KO on depression and cognitive function. The cognitive and antidepressant-like effects of KO were assessed after orally administering krill oil at two doses (1.25% or 2.5% of the daily ration of food) for 7 weeks to adult male and female Wistar-Unilever rats. The Aversive Light Stimulus Test (ALST) was applied for evaluating learning acquisition and resignation/depression, and the Forced Swimming Test (FST) for depression. An Imipramine (20 mg/kg) group was included as a positive control. The effect of treatments on cognitive function and depression was measured by the ability of rats to discriminate between active and inactive levers controlling the light cycle and their lever pressing activity. The FST assessed the immobility time of rats exposed to inescapable water. On the basis of our experimental conditions, the results indicate that active components in KO facilitate learning and memory processes and provide antidepressant-like effects. However, in both the ALST and the FST, the dose of KO 2.5 seemed to not be optimal for females.

Very long chain polyunsaturated fatty acids (VLC PUFAs) protect both neural- and retinal-derived cells from age-related damage

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Very long-chain polyunsaturated fatty acids (VLC-PUFAs) are present in the membrane phospholipids of the retina and brain in relatively small quantities, yet their function is largely unknown. In this study we sought to determine whether VLC-PUFA supplementation was beneficial to either healthy neuronal and retinal cell populations or to these same cell types after age-related insults. Pretreatment with select individual VLC-PUFAs generally inhibited cell death and promoted mitochondrial function when measured after hypoxic or glutamate insults to primary hippocampal neurons. However, these effects were more robust after glutamate exposure than after hypoxia, and the VLC-PUFAs had no appreciable benefit to healthy, unstressed hippocampal neurons. Conversely, equivalent experiments in a human cell line of the retinal pigmented epithelium (ARPE-19), suggested that VLC-PUFA pretreatment was beneficial to healthy, unstressed cells. Yet, only two of the five VLC-PUFAs tested, the 28:6n-3 and 34:6n-3 fatty acids, enhanced the viability of ARPE-19 cells during exposure to oxidative stress. In a related set of assays, we compared the native expression of VLC-PUFAs in fetal rat brain, ARPE-19 cells, bovine retina and maternal rat retina. Tissue differences were apparent, and the expression of VLC-PUFAs was very low compared to more prevalent fatty acids. Of the five VLC-PUFAs tested in the experiments described, only 28:5n-3 was expressed in all four tissue types, and significant species differences in the expression

of VLC-PUFAs were detected in the retina. The viability data and lipid profiles suggest that the human retinal pigmented epithelium may be deficient in most VLC-PUFAs and that the greatest benefits might be derived from 34:6n-3 supplementation. Overall, the current findings suggest that VLC-PUFA supplementation is generally beneficial to both neuronal and retinal cell populations.

Increased oxidative stress in LDL and HDL from patients with the metabolic syndrome or type 2 diabetes. Impact on platelet aggregation

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Metabolic syndrome (MetS) and type 2 diabetes (T2D) are associated with oxidative stress and increased risk for cardiovascular disease. Increased platelet activation occurs in MetS and T2D, and may represent a key contributing factor in the process of atherothrombosis.

The objectives of the present study were to assess whether low-density lipoproteins (LDL) and high-density lipoproteins (HDL) from MetS or T2D patients are oxidatively modified and to determine their effects on platelets. Compared to LDL from healthy subjects, LDL from MetS and T2D patients contained lower proportions of linoleic acid in phosphatidylcholine and cholesteryl esters and lower ethanolamine plasmalogen levels. Increased concentrations of hydroxylated fatty acids issued from linoleic acid (hydroxy-octadecadienoic acids, HODEs) and malondialdehyde were found in LDL from both patient groups. HDL from MetS or T2D patients were also oxidatively modified, as shown by decreased proportions of linoleic acid in phosphatidylcholine and cholesteryl esters, increased HODE concentrations, and decreased alpha-tocopherol concentrations, compared with HDL from healthy subjects. Pre-incubation of control platelets with LDL from MetS or T2D patients resulted in stimulation of platelet aggregation in response to sub-threshold concentrations of collagen, whereas LDL from healthy volunteers had no effects. By contrast, addition of HDL from both patient groups to control platelets inhibited collagen-induced platelet aggregation.

In conclusion, our data show that the occurrence of MetS, with or without T2D, is associated with increased oxidative stress in both LDL and HDL. Whereas oxidized LDL from these patients potentiated platelet aggregation, oxidized HDL showed a protective role against platelet aggregation and might possess anti-thrombotic properties despite oxidation.

Association between the levels of arachidonic fatty acid in human milk and weight gain in exclusively breastfed children

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Obesity prevalence has been increasing in childhood. Arachidonic fatty acid (AA), a naturally abundant fatty acid (FA) of the n–6 series is involved in the maturation of pre adipocytes during development of adipose tissue. AA is converted into prostacyclin, which stimulates adipose differentiation of primary preadipocytes in rodents and humans. We investigated associations between long chain polyunsaturated fatty acids (LCPUFA), with emphasis on AA, present in breast milk of Brazilian mothers and anthropometric variables of exclusively breastfed infants. The participants consisted of 71 mothers and their infants enrolled in a health center in Rio de Janeiro. The FA of total lipids from milk samples collected were identified and quantified by gas-liquid chromatography. Parameters were used for weight (w), head circumference, height (H) and age (A) and calculated the W/A, W/H, H/A, body mass index (BMI)/age and the weekly weight gain. Approximately 86% of infants were classified eutrophic. Taken together the percentages corresponding to the "risk of overweight" and "overweight", 21,1% and 9,8% of infants fall into this category, considered the indices W/H and BMI, respectively. The median levels of AA in milk were 0.57%. The average weekly weight gain differed among infants (male and female). The levels of AA in milk were positively correlated with weight gain of female infants, and the boys, only when exposed to higher concentrations of this fatty acid. It is suggested that AA is able to influence the weight gain of infants, and female children are more susceptible to the adipogenic effects associated with this fatty acid.

Effect of red wines with different antioxidant activity on the oxidative stress of rats submitted to a hyperlipideamic diet

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Phenolic compounds are associated to antioxidant proprieties in red wines, but their content varies according to the grape variety, geographical origin, winemaking techniques and vintage. It is not known if wines containing different in vitro antioxidant activity would be able to promote a higher or lower defense against oxidative stress in vivo. Thus, in the present study, Wistar rats were fed with a hyperlipidaemic diet (30% fat) for 30 days to induce inflammation and oxidative stress, and received a supplementation by gavage (770.0 – 1,360.00 µL/day) containing water and three red wines samples presenting high, medium and low in vitro antioxidant activity measured by ORAC methodology. Fatty acids composition, and biomarkers of inflammation and oxidative stress were determinate in plasma, liver and brain. The hyperlipidaemic diet promoted a replacement of linoleic acid (C18:2, n-6) by oleic acid (C18:1, n-9), reducing the proportion of polyunsaturated fatty acids (- 26%). The wines supplementation did not change the fatty acids proportion in plasma after 30 days under hyperlipidaemic diet. A lower concentration of liver malondialdehyde and higher ORAC values in plasma were observed in the animals supplemented with the wine containing the highest antioxidant activity. A significant correlation was observed between in vitro and in vivo antioxidant activity measured by ORAC (+ 0.49, p=0.011), and between in vivo antioxidant activity and C reactive protein (- 0.39, p= 0.049). Our preliminary results suggest that wines containing higher and lower in vitro antioxidant activity module the inflammation and oxidative stress in a different manner, being this response dependent on the biological sample and respective biomarker.

Concentration of Docosahexaenoic Acid (DHA) from Tuna Fish Oil by Lipase Catalyzed Hydrolysis and Selective Esterification

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Docosahexaenoic acid (DHA) is one of the most useful polyunsaturated fatty acid (PUFA) with pharmaceutical potential and important for the prevention & control of various human diseases and disorders such as cardiovascular disease, inflammation, allergy, cancer, immune response, diabetes, hypertension and renal disorders. It has been found to be very useful in treating the children affected by Dyslexia and Dyspraxia. DHA is also known as "brain food" as it is highly concentrated in the membranes of brain cells and retinal cells of eye. DHA plays an important role in the regeneration of the visual pigment rhodopsin, which has a critical role in the visual transduction system that converts light hitting the retina to visual images in the brain. Natural source of DHA is fish oil and tuna fish oil contains 25% DHA and it is one of the richest sources of DHA.

In the present investigation, concentration of DHA by lipase catalyzed hydrolysis of tuna fish oil and selective esterification of fatty acids, has been done. Candida rugosa lipase has been used for non-selective hydrolysis of tuna fish oil and free fatty acids has been separated from the reaction mixture by solvent extraction. Rhizopus oryzae lipase has been used for selective esterification of fatty acids other than DHA, obtained after hydrolysis of tuna fish oil. In 24 hrs, 86% hydrolysis has been obtained. Using Rhizopus oryzae lipase in 24 hrs, 76.17% esterification has been obtained resulting to 87% DHA concentration in free fatty acid fraction.

Key words: Tuna fish oil, docosahexaenoic acid, hydrolysis, esterification, Candida rugosa, Rhizopus oryzae.

Molecular cloning and biochemical characterization of a cholinephosphotransferase from Phytophthora infestans

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Cholinephosphotransferase (CPT) is a membrane-associated enzyme catalyzing the synthesis of phosphatidylcholine (PC) from diacylglycerol (DAG) and CDP-choline. Here, we report the identification of a cDNA (PiCPT) from Phytophthora infestans, a fungal pathogen of potato that can produce a high level of very long chain polyunsaturated fatty acids (VLCPUFAs), such as arachidonic acid (ARA, 20:4n-6). The predicted protein sequence PiCPT shares 26% amino acid identity to Saccharomyces cerevisiae

CPT with a conserved CDP-alcohol phosphotransferase motif. In vitro assays using PiCPT enzyme expressed in a yeast double mutant (CPT/EPT) showed that it could catalyze the synthesis of PC from 18:1-DAG and CDP-choline, indicating PiCPT codes for a functional cholinphosphotransferase. Substrate specificity assays showed that it preferred VLCPUFA-containing DAGs as substrates and the highest activity was found on ARA-DAG, followed by DHA-DAG, di18:2-DAG and di18:1-DAG. This is the first report describing a CPT gene (PiCPT) cloned from VLCPUFA-producing microorganisms which shows substrate specificity to VLCPUFAs, implying it might play a role in the production of these fatty acids by facilitating acyl-trafficking between phospholipid and neutral lipid pools.

The effects of omega-3 fatty acids supplementation on lipid profiles and insulin sensitivity in patients with schizophrenia: a randomized double-blind placebo-controlled study

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Introduction: Patients with schizophrenia had a high prevalence of metabolic syndrome. Omega-3 polyunsaturated fatty acids (PUFAs) supplementation may reduce triglyceride level and have a possible role in modulating insulin secretion in general population. Very few reports have investigated the effects of omega-3 PUFAs on lipid profiles and no report on glucose metabolism in patients with schizophrenia until now.

Methods: A 12-week, randomized, double-blind, placebo-controlled study was carried out to compare the effects of omega-3 PUFAs (6.8 g/day) against placebo (olive oil) on lipid profiles and insulin sensitivity in patients with schizophrenia. The laboratory measurements, including insulin, leptin, glucose, triglyceride, cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were assessed in series. Insulin sensitivity was calculated using the insulin/glucose ratio and homeostasis model assessment.

Results: Fifty-six participants were included in the analysis. Within group comparisons found that after 12 weeks' supplementation HDL was significantly increased and there was a trend toward to decreased triglyceride levels in the omega-3 group; however, there was no significant change in any lab measurements in the placebo group. In mixed model analyses, only insulin levels were found to be higher in the omega-3 group compared to the placebo group.

Discussion: In this study, we found a possible favorable lipid change in patients with schizophrenia after omega-3 PUFA supplementation, which were compatible with other studies. The increased insulin levels in the omega-3 group may represent a compensatory secretion of insulin due to non-significant elevated glucose levels (106.4 ± 38.3 to 113.7 ± 43.8 mg/dl) in their participants. Previously, a deterioration of glycemic control in people with consumption $\geqq$ 10g/day of fish oil has been suggested, while recent studies with doses of 1-2 g/day of fish oil showed no such deleterious impact. Whether high-dose n-3 PUFAs have impact on glucose homeostasis in patients with schizophrenia need further investigations.

Importance of cyclooxygenase activity for the expression of multiple drug resistance proteins (MDRPs) in human glioma

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Introduction: The highly malignant tumour glioblastoma multiforme is difficult to cure because of its resistance to chemotherapy. A well-established cause of multidrug resistance (MDR) involves the increased expression of members of the ATP binding cassette (ABC) transporter superfamily, many of which transport chemotherapeutic compounds from cells. The possible relationship between cyclooxygenase (COX) and MDR proteins (MDRPs) has been studied in several cancers and in some there is a positive relationship. The present study aimed to analyse the possible relationship between COX-1 and COX-2 and the expression of MDRPs in gliomas. The following ABC transporters were studied: MDR1, MRP1, MRP2, MRP3, MRP4, MRP5 and MRP6.

Methods: The human glioma cell lines A172, T98G, U87MG, U138MG and U251MG were used. The cells were analysed by PCR to verify the mRNA expression of COX-1 and COX-2. After determining the expression of COX-1 and COX-2 the cell lines U251 and U138MG were used in further studies as the cell

line U138MG expressed low levels of COX-1 while U251MG expressed both COX-1 and COX-2. After treatment with COX-1 (SC-560) or COX-2 (NS-398) inhibitors the expression of MDR and MRPs1-6 was also determined.

Results and Discussion: The results showed constitutive expression of COX-2 in U138MG and U251 cells, as well as the expression of all the MDRPs studied (MDR1 and MRPs1-6). However, U138MG, which expressed low levels of COX-1, expressed very low levels of MDR1. Inhibition of COX-1 and 2 caused a decrease in the expression of several MDRPs including MDR1, MRP1 and MRP4 in both cell lines. It is possible that the reduction in prostaglandin production may be linked to decreased MDRP expression since they are substrates for MDRPs including MDR1, MRP2 and MRP4. The mechanism behind these changes is currently under investigation in our laboratory.

Modifying prostaglandin metabolism alters proliferation, apoptosis and migration in the T98G human glioma cell line

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Introduction: Glioblastoma multiforme (GBM) is one of the most common intracranial tumours and no treatment for GBM, including radiotherapy and chemotherapy, has yet been completely successful. GBM is characterized by rapid cell proliferation, migration, angiogenesis and reduced apoptosis. This study aimed to analyse the effect of altering prostaglandin metabolism in vitro upon proliferation, migration and apoptosis in the T98G human glioma cell line.

Methods: T98G human glioma cells were treated with Ibuprofen (IBP) (25μM and 50μM), PGE1 (10μM) or PGE2 (10μM) for up to 72 hours. After treatment cell proliferation (cell count and FACS), mitotic index and apoptosis (apoptotic nuclei stained with Hoechst 33342 or propidium iodide) were analysed. The migration activity was quantified using a wound healing migration assay and a transwell migration assay for up to 48 hours.

Results and Discussion: The effects of IBP were dose and time dependent and are expressed as % of control (p<0.05). We observed a significant decrease in proliferation after treatment with 50μM IBP (54%) and a decrease in mitotic index (57%). Migration decreased in cells treated with 25μM IBP (40%) and 50μM IBP (74%). Finally, the apoptosis assay showed increased apoptosis after treatment with 50μM IBP (167%). The effects of PGE1 treatment were also dose and time dependent, with significant increases in cell proliferation (37%) and migration (33%) at 10μM. More pronounced effects were seen for 10μM PGE2 with increases of 45% and 66% for proliferation and migration, respectively. The present study demonstrated that treatment with IBP reduced the proliferation and migration of T98G cells, whereas the addition of exogenous PGE1 or PGE2 increased these processes. These data show the influence of PGE1 and PGE2 on important processes for the development of GBM including cell proliferation, migration and apoptosis.

The development of a universal method to quantify the choline-containing compounds in foods, tissue and extracts

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Choline/phosphatidylcholine (PC) functions in the body in brain development, lipid transport and organ function. It can be synthesized in the body by adult humans and animals but it has been established that the fetus is unable to synthesize PC. Until very recently, when adequate dietary intakes (AI) were established by the Food and Nutrition Board (a joint venture of Health Canada and the FDA), dietary choline has been somewhat overlooked. There is still insufficient data to establish a dietary requirement during pregnancy, beyond an estimated AI.

In order to perform meaningful studies on dietary intakes of choline and to conduct animal trials to probe its impact in areas such as maternal and infant immune and intestinal health, it is essential to develop sophisticated analytical techniques that can identify and reliably quantify the diverse structural forms and

amounts of choline present in a wide variety foods and in tissue samples. Recently, we have published a "universal" method which allows us to quantify the 11 important compounds or compound classes containing choline in a single LC/MS/MS experiment using standards we have synthesised. In this study we report on some new developments to this method aimed at getting the higher throughput we require for nutritional studies and for establishing mean levels present in a range of local foods. Further, we describe the validation of the method including comparisons to data from 31P NMR experiments.

Fast foods consumed by Brazilian college students are rich in saturated and trans fatty acids

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Recent research about familiar costs among Brazilian population indicated frequent intake of salty snacks, a kind of preparation obtained by the cooking of dough prepared with flour. It can be stuffed or not and are generally rich in fat, specially saturated fatty acids (SFA) and trans fatty acid (TFA), which are associated with cardiovascular disease. The present study aimed to evaluate fatty acid profile of fast-foods commonly eaten by students in a Brazilian university. The salty snacks were selected according to results of a previous study carried out in order to evaluate food habits among college students. Total lipids were extracted from 3 samples of each 10 types of snacks, according to the procedure published by Bligh & Dyer. The fatty acids methyl esters were prepared by methylation of fatty acids and analyzed by using gas chromatograph equipped with flame ionization detector. The chromatogram peaks were assigned on the basis of comparison with reference standards and the integrated peak areas were used to assign the percentual composition. Twenty-seven fatty acids were identified and quantified, being classified as 13 SFA, 6 monounsaturated fatty acids, 6 polyunsaturated fatty acid and 2 TFA. Relative high contents of TFA, mainly elaidic acid (C18:1n9t), were observed. As this fatty acid is found in hydrogenated vegetable fat, it is possible that it is often added to the snacks dough. It is known that these fatty acids are present in bakery foods; however, the content of this compound found in these snacks was higher than the expected. In conclusion, the high content of lipids, especially SFA and TF, of the salty snacks analyzed in this study indicates that the consumption of these can significantly increase the risk of cardiovascular disease, which represents the major cause of death worldwide.

Keywords: trans fatty acids, college students, fast foods

Arachidonic acid modulates permeability of human brain microvessel endothelial cells

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Background: Arachidonic acid (AA), an omega-6 polyunsaturated fatty acid, is a component of membrane lipids in the body and is particularly abundant in the brain, where it also plays the role of as second messenger in cellular signaling pathways. Dietary AA can lead to the production of both pro- and anti-inflammatory molecules. The effect of AA on permeability of human brain microvessel endothelial cells (HBMEC) was examined.

Methods: HBMECs were grown to confluence on polycarbonate membrane inserts in Transwell® plates. The effect of AA on permeability of HBMECs was studied alone or in conjunction with cyclooxygenase inhibitors indomethacin, celecoxib and NS-398. Permeability from apical to basolateral medium was assessed using fluorescent dextran as marker. In addition, the effect of AA on PGE2 production and expression of prostaglandin E synthase, a key enzyme in the synthesis of PGE2, was determined.

Results: AA exposure resulted in a marked increase in permeability of the HBMEC monolayer over 30 min. This effect of AA on HBMEC permeability was blocked by the presence of cyclooxygenase inhibitors studied, celecoxib and NS-398 being most effective. Exposure of HBMEC to AA resulted in an increase in PGE2 production and PGE synthase mRNA expression.

Conclusions: AA increases permeability of HBMEC monolayers via an increase in PGE2 production.

The effects of n-3 long-chain polyunsaturated fatty acids on bone formation and growth factors in adolescent boys

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Bone mineral accumulation during childhood and adolescence plays an essential role in the prevention of osteoporosis. Animal studies indicate that n-3 long-chain polyunsaturated fatty acids (LCPUFA) increase bone formation, however no studies have examined this in growing humans. This study investigated if bone mass and markers of bone formation and growth were 1) associated with docosahexaenoic acid (DHA) status and 2) affected by fish oil supplementation, in adolescent boys. Seventy-eight healthy, slightly overweight boys 13-15 year-old boys were randomly assigned to breads with DHA-rich fish oil (1.1 g/day n-3 LCPUFA) or control for 16 weeks. Whole-body bone mineral content (BMC), bone area (BA), bone mineral density (BMD), plasma osteocalcin, and growth factors were measured at week 0 and week 16, as well as diet, physical activity, and erythrocyte n-3 LCPUFA status. Fish oil strongly increased erythrocyte DHA status (P=0.0001). No associations were found between DHA status and BMC, BA, BMD, or the markers of bone formation and growth at baseline. Furthermore, the fish oil intervention did not affect any of the outcomes, compared to control. However, dose-response analyses revealed a positive association between changes in DHA status and plasma insulin-like growth factor-1 (IGF-1) during the intervention (β=0.24, P=0.03, n=78). In conclusion, DHA status and fish oil supplementation were not associated with bone mass or markers of bone formation in adolescent boys, but the growth factor IGF-1 increased with DHA status.

Metabolism of conjugated fatty acids (CLA and CLnA) in human colon cell lines with different stage of transformation: challenges and chances

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Experimental models have contributed to an improved understanding of the genesis and the possibilities of intervention in diet-related diseases such as coronary heart disease, diabetes, and cancer. Several studies have confirmed various health benefits of CFAs (conjugated fatty acids) like conjugated linoleic acids (CLA) and conjugated linolenic acids (CLnA). This investigation focuses on the incorporation and metabolism of CFA in the adenomatous cell line LT-97 in comparison to the well-established colon adenocarcarcinoma cell line HT-29. The incorporation and metabolism of CFA with cis/trans and all-trans double bonds was compared to the best studied CLA isomer (c9,t11). Analysis of cellular fatty acids revealed a 2-fold higher incorporation of CFA like c9,t11-CLA (40 µM and 80 µM) in lipids of HT-29 cells compared to LT-97 cells (% FAME; HT-29; 40 µM =15.3 vs. HT-29; 80 µM = 28.1 and LT-97; 40 µM = 6.9 vs. LT-97; 80 µM = 14.6). Albeit, both cell lines differ considerably regarding culture and growth specificities. LT-97 cells showed more versatility and a greater capacity to metabolize c9,t11-CLA. Whilst the ratio of β-oxidized elongated conjugated dienoic (CD) showed an 8-fold difference between the cell lines (CD-C16:2/CD-C20:2; HT-29: 8:1; LT-97: 1:1), cellular lipids showed an equal percentage composition (% FAME CD-C16:2 + CD-C20:2; HT-29/LT-97; 40 µM = 0.8%, HT-29/LT-97; 80 µM = 1.5%). Notably, the conversion of CLnA to CLA (c9,t11,t13-CLA to c9,t11-CLA) and an interconversion of a CLA isomer (t11,t13-CLA to c9,t11-CLA) was shown in both cell lines.

Although, LT-97 cells incorporated lower amounts of CFA, the cell culture might represent physiologic conditions to a better extent compared to HT-29 cells due to a more balanced FA metabolism. Thus, LT-97 cells are useful tools investigating physiological modes of metabolisms as well as impact of beneficial food components such as CFA in early stages of colon cancer.

Preterm Infant Long-Chain Polyunsaturated Fatty Acid (LCPUFA) Consumption via Human Milk Improves Plasma Docosahexaenoic Acid (DHA) Levels

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Background: Preterm delivery disrupts in utero accretion of LCPUFA. Postnatal LCPUFA status is in part dependent upon the LCPUFA content of the human milk fed to preterm infants.

Objective: To evaluate the relationship between enteral intake of LCPUFA via fortified human milk and plasma LCPUFAs in preterm infants.

Methods: Posthoc analyses were performed on data from preterm infants (BW <1,250 g) in a prospective randomized trial who were fed human milk fortified with either liquid HMF (providing 12 mg DHA + 20 mg arachidonic acid (ARA) when added full strength to 100 mL human milk, LHMF) or powder HMF (with no added DHA and ARA, PHMF) for 28 days. Infant plasma phospholipid (PPL) fatty acids on study day 28 were quantified by capillary column gas chromatography. Maternal consumption of fish and/or DHA supplements (1 time per week) during the last 12 weeks of pregnancy was recorded. Mean PPL LCPUFA were determined for infants: (1) whose mothers did not consume fish or DHA supplements and were fed PHMF; (2) whose mothers consumed fish and/or supplements and were fed PHMF; (3) whose mothers did not consume fish or supplements and were fed LHMF; and (4) whose mothers consumed fish and/or supplements and were fed LHMF.

Results: Milk % DHA was lower among mothers who did not consume fish or DHA supplements (0.27±0.14, mean±SD) vs. those who did (0.39±0.23). Infants who received DHA from LHMF and had higher maternal DHA consumption had the highest PPL % DHA (3.82±0.89), while infants who were fed PHMF and whose mothers did not consume fish or DHA supplements had the lowest PPL % DHA (3.05±0.42).

Conclusion: Infants fed more DHA in human milk, from maternal DHA intake and LHMF, had the highest PPL DHA.

Eicosapantaenoic acid moderates MeHg-induced metabolic abnormalities via COX-2 inhibition in C57Bl/6J mice fed high fat diet

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The neurotoxicity of methymercury (MeHg) has been well known, but the knowledge of the MgHg-induced metabolic abnormality is still poor. The present study investigated the effects of MeHg on systemic metabolic properties in the C57Bl/6J mice fed with high-fat for 6 weeks. Moreover, we hypothesized that eicosapentaenoic acid (EPA) as COX-2 inhibitor might has protective effects against these MeHg-induced metabolic abnormalities. Therefore, the effects of the combinations of MeHg and EPA, or indomethacine, a COX-2 inhibitor drug, were also tested. The results indicated that the feed intake, liver weight and plasma levels of triglycerides and free fatty acids were comparable among groups. However, as compared with the control, 10 ug/kg MeHg in high fat diet led to higher body weight and more lipid deposit in adipose tissue, also elevated plasma levels of cholesterol, prostaglandin E2 and TNF-a, whereas impaired the catabolism during fasting. Moreover, a tendency of insulin and leptin resistance was also seen in the MeHg group. Of note, dietary supplement of 3% EPA, or 16 mg/kg indomethacine moderated the above metabolic abnormalities, but did not change the Hg residue in liver, mucle, adipose tissue and feces. Gene expression assay also suggested that EPA and indomethacine improved metabolisms of fatty acids and glucose, also suppress systemic inflammation. There was non-signficant difference between EPA and indomethacine groups in all tested parameters. In conclusion, MeHg in high fat diet can cause severe metabolic abnormalities, and EPA may alleviate the metabolic toxicity of MeHg, at least partly via COX-2 inhibition pathway.

Atherosclerosis: assessment of inflammation and its resolution on a primary human macrophage-derived foam cells model

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Introduction: Atherosclerosis is an inflammatory disease, which involves the macrophages present in the arterial intima. Those macrophages become foam due to oxidized Low Density Lipoprotein (oxLDL) uptake. The aim of this work was to study the inflammatory profile of primary human macrophage-derived foam cells. We have focused our attention on the capacity of cells to metabolize polyunsaturated free fatty acids (PUFAs) into inflammatory and pro-resolving molecules. A set of genes linked to inflammation and its resolution were also evaluated.

Methods: For this purpose, monocytes from human donor were differentiated into macrophages, which were then incubated in the presence of oxLDL. We have focused our attention on the capacity of macrophage-derived foam cells to metabolize PUFAs into inflammatory and pro-resolving molecules after stimulation with a TLR-4 ligand. Bioactive lipids were quantified by using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology. Thanks to a microarray, we have assessed the expression of specific genes implied in the inflammation and in PUFAs metabolism.

Results: We have shown that in presence of oxLDL, the cells produce more inflammatory (TXB2, PGE2) and oxidative stress mediators (5-oxo-ETE) than the cells that have not received oxLDL. On the other hand, the cells have also the capacity to produce pro-resolving mediators such as resolvins and maresin. By measuring mRNA expression we have shown that COX-2 and 15-LOX were strongly increased. On the contrary, 5-LOX and FLAP were down-regulated. No effect on 12-LOX was measured. We have also observed an increase of IL-1b, TNFa, MMP-9 genes and a decrease of TGF-ß2 mRNA expression.

Conclusions: Taken together these results demonstrate that we have developed a human model of macrophage-derived foam cells, which correspond to macrophages present in atheroma (M1 phenotype). This model is a valuable tool to screen the effects of compounds on the resolution of inflammation in an atherosclerotic plaque.

C-reactive protein concentration and total white blood cell count in homozygous sickle cell disease are not influenced by Omega-3 fatty acid supplementation

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Background: Chronic inflammation is a one of the major features of sickle cell disease (SCD). Studies have demonstrated that sickle patients, even at steady state, have significantly reduced levels of the omega-3 fatty acids, eicosapentnoic (EPA) and docosahexanoic (DHA) in plasma and blood cells. This abnormality was unrelated to low intake. EPA and DHA are known precursors of anti-inflammatory mediators.

Objectives: the aim was to assess the effect of omega-3 fatty acid supplementation on the inflammatory markers, C-reactive protein and total white blood cell count.

Procedure: Steady-state homozygous sickle cell patients (HbSS) supplemented with DHA and EPA for two years (n=25), their unsupplemented counterparts (n=22) and healthy controls (HbAA, n=12) matched for age (2-18 years), gender and socio-economic status were studied. The supplemented group received one (2-4 years old), two (5-10), three (11-16) and four (≥ 17) omega 3 fatty acid capsule containing 277.8 mg DHA and 39.0 mg EPA. Blood taken from the three groups were analysed for red blood cell DHA and EPA, C-reactive protein (CRP) and total white blood cell (TWB) count.

Results: The supplemented group had higher levels of DHA and EPA ($p<0.001$) compared with the un-supplemented and healthy controls. There was no difference in plasma C-reactive concentrations (Median=1.6, IQR=1.8 VS Median=1.9, IQR=2.8 mg/l non-supplemented, $p>0.05$) and total white blood counts (Median=14.2 x103/µL, IQR=7.3 x103/µL VS Median=13.1 x103/µL, IQR=7.8 x103/µL non-supplemented, $p>0.05$) between the supplemented and un-supplemented patients. Both groups of patients had higher concentrations of C-reactive protein and total white blood cell count compared with

their healthy controls (Median=0.09, IQR=0.05mg/l, p<0.001) and (Median= 5.4 x103/µL, IQR=2.2 x103/µL, p<0.001) respectively.

Conclusion: This pilot study suggests that supplementation with DHA and EPA does not influence CRP and TWB in patients with homozygous sickle cell disease. There is a need for further well –powered study with patient on both high DHA and EPA supplements.

Lipid Characterization of Cells by Liquid Extraction Surface Analysis Mass Spectrometry

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Liquid extraction surface analysis (LESA) is a recent technique allowing for the direct extraction and analysis of analytes from a variety of surfaces. LESA is particularly useful for lipid analysis as tissue samples and cells can be analysed directly without the need for prior lipid extraction. In this study LESA was used for the rapid identification of lipids from cells present on microscope slides. LESA analysis of C2C12 and PC12 cells allowed the detection of a range of lipid classes including, phospholipids, sphingolipids and cholesterol esters. Additionally, principal component analysis allowed the rapid differentiation of the C2C12 and PC12 cells based on their lipid profiles.

The use of LESA for single cell detection will also be demonstrated. Cells were deposited onto glass slides using a custom made inkjet printer with the capacity to deposit a single cell at a known location on the substrate. LESA analysis of a single cell is shown to readily detect a variety of phosphatidylcholines. Single cell analysis by LESA will be used to compare the lipid profile of individual cells from the same culture and across different cell types. This study demonstrates that LESA is a highly sensitive technique allowing for the lipid profiling cells, including single cells. Unlike previously used laser based and fine tip extraction methods for single cell analysis, LESA allows for the analysis of lipids from the entire cell volume in a single step.

Zebrafish FABP1b and FABP2 regulation and their functional role in intestinal lipid absorption

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Dietary fatty acids (FAs) are absorbed by the enterocyte. Once inside the cell, FAs are bound reversibly by transporters like fatty acid binding proteins (FABPs). The precise role of each FABP type in enterocyte remains unclear. In zebrafish as in mammals, the proximal third of the intestine is the major site of fat absorption. We have recently demonstrated that zebrafish enterocytes strongly expressed FABP1b and FABP2 mRNAs.

The aim of the work was to study the regulation of FABP genes after feeding to describe the intracellular FABPs distribution inside the enterocyte.

Gene expression pattern was performed by real time quantitative RT-PCR (QPCR) in larvae and adult intestinal tissues. Antibodies raised against zebrafish FABP1b and FABP2 recombinant proteins were used in immunohistological analyses and electron microcopy immunodetection with the secondary antibody coupled to gold particles.

QPCR revealed a pretranslational up-regulation of both genes after feeding at 15 dpf. This regulation appeared to be modulated by food composition. This up-regulation was observed in the adult intestine by QPCR for FABP2 when normalized with the reference gene ef1alpha. Immunodetection on adult intestine demonstrated FABP1b and FABP2 localized in the enterocyte, at the microvillosities and cytoplasmic level and in some cases in the nucleus. A colocalization was found with fatty acids labeled with Bodipy. This last finding suggests that FABPs may be used as transcription factors after translocation the fatty acids inside the nucleus.

These data indicate that these FABPs play common and also specific roles in lipid-metabolic processes in the zebrafish gut.

The Hypolipidemic Effect of an Ethyl Ester of Algal-Docosahexaenoic Acid in Dogs

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MATK-90 is a concentrated ethyl ester of DHA (~900 mg/g) manufactured from microalgal DHA oil (DHASCO®). MATK-90 (600, 1300, 2500, 5000 mg/kg/day for 28 days) dose-dependently reduced triglyceride (TAG) and cholesterol levels in Wistar rats fed a high-fructose diet (Ryan et al., 2009). Recently, a 9-month toxicity evaluation including a 2-month recovery period was conducted in dogs to provide a more comprehensive test of safety for MATK-90. MATK-90 (150, 1000, and 2000 mg/kg/day) was administered once daily via oral gavage to beagle dogs (5 animals/sex/dose). Control animals received corn oil at the same volume. Dogs were fed Teklad Dog Diet 2025 containing 25% of protein and 10% of fat. Lipids values were within the historical control normalized ranges. Blood samples for clinical chemistry evaluation were collected before the study start (Day -5) and on Days 23, 92 and 274 (at study termination) as well as at the end of the recovery period (Day 330). MATK-90 administration led to a decrease in TAG levels compared to pre-study levels at all three doses and in both genders by Day 23. The levels stayed about the same throughout the study duration and rebounded back to the pre-study values at the end of the recovery period (Day 330). TAG levels were statistically significantly reduced compared to control at all three doses and in both genders during the study period (Days 23, 92, and 274). Interestingly, the effect was not dose-dependent: the dose increase from 150 mg/kg/day to 1000 and 2000 mg/kg/day did not provide any further benefits for TAG lowering. Total cholesterol levels were also reduced by MATK-90 administration during the study, although in males a statistically significant effect was observed at all dose levels (again, with no dose-response) and in females only at the mid- (1000 mg/kg/day) and high (2000 mg/kg/day) doses.

Very high prevalence of vitamin A, E and D deficiencies in very low birth weight Tunisian infants

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Introduction and aims: Premature infants are exposed to a high risk of nutritional deficit that increases the risk of diseases in neonatal period and later. This study was aimed to determine the prevalence of deficiencies in vitamins A (VAD), E (VED) and D (VDD) in very low birth weight (VLBW) Tunisian infants and to seek for their relationship with obstetrical and neonatal poor outcomes.

Methods: The study included 607 VLBW infants (birth weight <1500 g), admitted in neonatology service of The Maternity and Neonatology Center (Tunis, Tunisia). Plasma vitamin A and E were assessed by HPLC and vitamin D was assessed by radioimmunoassay. Moderate vitamin deficiencies were considered for plasma vitamin A < 0,70 µmol/l, vitamin E < 7 µmol/l and vitamin D < 25 nmol/l.

Results: A moderate vitamin deficiency was observed in 75.9%, 71.3% and 65.2% of infants for VAD, VED and VDD, respectively. VAD was more frequent in infants with respiratory distress [77.8% vs. 66.4%; OR (95% CI), 1.78 (1.01-2.88)]. VED was more frequent in infants with uterine growth retardation [76% vs. 63%, 1.86 (1.19-2.90)] and those with maternal gestational hypertension [77.2% vs. 68.4%; 1.56 (1.01-2.44] or gestational diabetes [90.9% vs. 71.4%; 4.01 (1.01-17.1)]. VDD was more frequent in infants with maternal gestational hypertension [84% vs. 64.5%; 2.89 (1.36-6.40].

Conclusion: Vitamins A, E and D deficiencies are highly frequent in VLBW Tunisian infants. These deficiencies were associated with obstetrical complications and would increase the risk of infant morbidity and mortality.

Semen abnormalities are associated with decreased docosahexaenoic acid and increased oleic acid contents in Human spermatozoa

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Introduction and aims: Fatty acid composition of spermatozoa could play an important role in their structure and function. This study was aimed to establish relationship between spermatozoa fatty acids and semen characteristics and to look for a possible alteration of this profile in sperm abnormalities.

Methods: The study involved 45 men attending for couple infertility. The semen was collected after 2 to 4 days of abstinence, centrifuged, and the spermatozoa pellet was washed with isotonic NaCl and stored at

- 80°C. Fatty acid profile was analyzed by gas chromatography. The WHO criteria were adopted to define normal sperm and different sperm abnormalities.

Results: Normal sperm is particularly rich in saturated fatty acids (64.5%), mainly palmitic acid (41.8%) and in docosahexaenoic acid (DHA) (17.1%). Compared with normal sperm, oleic acid (OA) level was increased (9.07 ± 5.44 vs. 4.46 ± 2.43) and DHA level was reduced (11.04 ± 9.90 vs. 17.1 ± 11.3) in pathological sperm. Spermatozoa number and motility were correlated positively with DHA content and negatively with AO content, whereas the number of atypical spermatozoa showed the inverse correlations.

Conclusions: A high DHA content would contribute to the optimal functioning of spermatozoa. Sperm abnormalities are associated with a reduction in DHA and an increase in AO, probably related to excessive oxidation of polyunsaturated fatty acids. Thus, a diet rich in DHA and antioxidants could help improving the quality and the fertilizing ability of sperm.

Effects of the quality of dietary lipid during early pregnancy in rats on fatty acid composition of adipose tissue, colostrum and milk, and repercussions on pup's development

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Fat accumulation is one of the most common characteristics of pregnancy. The increase in maternal fat deposits occurs during the first two-thirds of gestation. It declines or even stops during the last third, (the latter) corresponding to the most accelerated lipolytic activity of adipose tissue, coincident with maximal fetal growth. Essential fatty acids and their long-chain unsaturated derivatives are crucial for fetal growth and development, their requirements increase during pregnancy, particularly in the last third. To determine if the composition of essential fatty acids n-6 and n-3 could be modified in adipose tissue, pregnant Sprague-Dawley rats received soybean (SO), olive (OO), fish (FO) and linseed (LO) oil diets from conception to d12 of gestation (early diets) and standard diet thereafter. At d12 and d20 the lipoprotein lipase (LPL) activity was evaluated in maternal lumbar, perirenal and periuterine adipose tissues (ATs). Fatty acid (FA) profile was determined in maternal lumbar AT (LAT), in milk and in pup´s plasma and brain. LPL activity was higher in the 3 ATs at d12 than d20, all groups presenting hypertriglyceridemia at d20. At d12, n-3 polyunsaturated fatty acids (PUFAs)-rich diets resulted higher LPL activity and incorporation of n-3 PUFAS into LAT. At d20, FA profile in maternal LAT was similar to early diets. Compared to mature milk, colostrum presented FA profile more similar to early diets, reflected also in FA composition of pup´s plasma. FO and LO pups had higher proportions of n-3 PUFAs in plasma. Brain phospholipids had higher DHA in FO and lower AA in LO. Results show that specifics dietary FA in early pregnancy modulates lipid metabolism in anabolic and catabolic stages of gestation and the provision of LC-PUFAS in milk and brain pups.

Changes in the composition of dietary maternal fatty acids on levels of fatty acids in adipose tissue of rats during the first half of pregnancy

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Fat accumulation is one of the most common characteristics of pregnancy. The increase in maternal fat depots occurs during the first two-thirds of gestation, it declines or even stops during the last third, (the latter) corresponding to the most accelerated lipolytic activity of adipose tissue, coincident with maximal fetal growth. Essential fatty acids and their long-chain unsaturated derivatives are crucial for fetal growth and development, their requirements increase during pregnancy, particularly in the last third. To determine if the composition of essential fatty acids n-6 and n-3 could be modified in adipose tissue, pregnant Sprague-Dawley rats were fed semi purified diets added with either soybean oil, olive oil, flaxseed oil, fish oil or palm oil. A total of 8–12 rats were studied by group. The fatty acid composition of the lumbar adipose tissue of each group appeared to be close to the fatty acid composition of the oil added to diets eaten in early gestation. At day 12 and 20 of gestation, the fatty acids in maternal lumbar adipose tissue were similar in all groups. However, lower percentage of n-6 and n-3 fatty acids in palm and olive fed groups and lower levels of n-6 fatty acids in fish fed group were observed in adipose tissue

in early pregnancy (to) compared with non-pregnant rats. (This) These differences in fatty acids incorporation could play an important role in the specific transfer of these important fatty acids to the fetus.

Lipid profile of infant and young children formulas marketed in Mexico with emphasis on trans fatty acids

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Background: Omega-3, Omega-6 and essential fatty acids (EFA) as linoleic acid (LA) and linolenic acid (ALA) play an important role in the development of infants and young children. On the other hand, trans fatty acids (tFA) are implicated in poor fetal and infant early growth, and may compete with LA for desaturation. No evidence of lipid quality of infant formulas marketed in Mexico is reported.

Objective: To assess the lipid quality of infant formulas and other milk products aimed for Mexican pediatric population under 5 years.

Procedure: 34 products aimed for infants and young children were purchased in Mexico City's supermarkets (stage 1(n=9): 0-6 months; stage 2(n=9): 6-12 months and stage 3(n=16): 1-5 years). Duplicate samples were treated for extraction of total lipid content with the modified Folch procedure. Fatty acid methyl esters were prepared using sodium methoxide and boron trifluoride and analyzed by gas-chromatography. 36 fatty acids (FA) were identified and their amounts calculated based on area(%w/w). Recommendations of different organizations (WHO, FAO, ESPGHAN) for the intake of fats in children were projected on their FA composition.

Results: LA and ALA levels in stages 1 and 2 formulas met the recommendations. Levels of arachidonic acid and docosahexaenoic acid were insufficient in 6 of 9 stage 1 formulas (≥ 0.35 and ≥ 0.2 %, respectively), while levels of both FA failed to meet this recommendations in 7 of 9 stage 2 formulas. Regarding stage 3 products, 60% exhibited an unfavorable ratio of unsaturated to saturated FA (≥ 2). In addition, 3 of them exhibited a greatly imbalanced ratio of omega-6/omega-3 FA (> 30). Finally, 27 samples had very low amounts of tFA (< 1%).

Conclusion: Few samples exceeded recommendations for tFA content. However, the levels of omega-3, omega-6 and EFA were found to be inadequate in most of the infant formulas and milk products analyzed.

Long-chain n-3 Polyunsaturated Fatty Acids of Marine Origin Reduce Fat Cell Proliferation in Dietary Obese Mice

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Background/Objective: Our previous study in mice showed that reduction of adiposity by long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) of marine origin was associated with both, a shift in adipose tissue metabolism and a decrease in tissue cellularity. The aim of this study was to further characterize the effects of LC n-3 PUFA on fat cell proliferation and differentiation.

Procedures: A murine model of inducible and reversible lipoatrophy was used, in which the death of mature adipocytes could be achieved in response to i.p. tamoxifen injection (aP2-Cre-ERT2 PPARgL2/L2 mice). Obesity was induced by feeding a high-fat diet (cHF) and, subsequently, mice were randomly assigned (day 0) to following groups: (i) mice injected by vehicle, i.e. "control" mice, and fed cHF; (ii) mice injected by tamoxifen, i.e. "mutant" mice, fed cHF; (iii) control mice fed cHF diet with 15% of dietary lipids replaced by LC n-3 PUFA (cHF+F); and (iv) mutant mice fed cHF+F.

Results: Mutant mice achieved a maximum weight loss within 10 days, followed by a compensatory body weight gain, which was significantly faster in the cHF as compared with the cHF+F mutant mice. Also in control mice, body weight gain was depressed in response to dietary LC n-3 PUFA. At day 42, body weights in all groups stabilized, with no significant differences in adipocyte size between the groups,

although body weight and adiposity was lower in the cHF+F as compared with the cHF mice, with a stronger effect in the mutant than in control mice. Gene expression analysis documented depression of adipocyte maturation during the reconstitution of adipose tissue in the cHF+F mutant mice.

Conclusion: Dietary LC n-3 PUFA could reduce both hypertrophy and hyperplasia of fat cells in vivo. Results are in agreement with the involvement of fat cell turnover in control of adiposity.

Functional link between arachidonic acid and endocannabinoids in the regulation of inflammation

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Arachidonic acid is a fatty acid involved in most, if not all physiological processes. Its metabolism into pro- and anti-inflammatory eicosanoids (e.g. leukotrienes, prostaglandins, lipoxins) either results in enhanced or decreased inflammation. Other bioactive lipids play key roles during inflammation. Among them are the endocannabinoids, which consist of a fatty acid linked to a molecule of glycerol or a molecule of ethanolamine. The resulting glyceryl-esters (e.g. 2-arachidonoyl-glycerol) and ethanolamides (e.g. arachidonyl-ethanolamide) have been linked to the regulation of inflammation by activating the specific G-protein-coupled receptors CB1 and CB2. Endocannabinoids are biosynthesized on demand and are hydrolyzed rapidly to fatty acids. While the pharmacological or genetic inhibition of cannabinoid receptors supports an anti-inflammatory role of endocannabinoids, the latter induce pro- and anti-inflammatory effects. We believe this is related to 1) their metabolism by eicosanoid biosynthetic enzymes; and 2) their hydrolysis into arachidonic acid and the subsequent synthesis of eicosanoids. The resulting lipidome consists of numerous bioactive lipids with either pro- or anti-inflammatory effects. Interestingly, while endocannabinoids can serve as a source of arachidonic acid, fatty acid intake modulates endocannabinoid levels in the tissues. Recent evidence supports this functional link between arachidonic acid and endocannabinoids as they play a key role in the regulation of inflammation. In this regard, it remains unclear whether we should enhance or reduce arachidonic acid levels/intake in order to limit the onset of inflammation and to promote its resolution. Key findings regarding the functional link between endocannabinoids and arachidonic acid will be presented.

Linoleic Acid Status of Adolescent Girls in Central Mozambique – ZANE-Study

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Traditional African diets are often high in carbohydrates and low in fat. This may compromise the intake of essential fatty acids. Adolescent girls who are still growing but likely to become pregnant may be especially vulnerable. Little is known on the fatty acid status of this group in Sub-Saharan Africa.

We have collected data on the diet and nutritional status of adolescent girls in Zambezia province, central Mozambique. Here we report the status of linoleic acid (LA, 18:2n-6) based on serum phospholipid fatty acid analyses. Mead acid (MA, 20:3n-9) and its ratio to arachidonic acid (AA, 20:4n-6) are used as markers of potential LA insufficiency.

This cross-sectional study was carried out in January-February 2010. Girls (n=262) aged 14 to 19 years (median 16 years) were studied in Quelimane city (urban area), as well as in the central areas (vilas) and rural villages of a coastal and an inland district. Non-fasting serum samples were collected, frozen and shipped to Europe for analyses. Serum phospholipids were separated by thin layer chromatography and fatty acid composition was analysed by gas-liquid chromatography.

Mean (SD) proportion of LA was 16.6% (2.8%) and the range (min–max) 10.3–24.7% of fatty acids. Mean proportion of MA was 0.39% (0.28%) and it ranged from undetected (i.e.<0.01% n=2) to 1.52%. LA and MA proportions correlated (r=–0.488, p<0.001). The ratio of MA to AA was on average 0.036 (0.031) and ranged from 0.007 to 0.192. LA was highest and MA lowest in Quelimane, followed by district vilas and rural villages. The ratio of MA to AA was highest in the inland rural villages and lowest in Quelimane.

These results indicate that LA status of adolescent Zambezian girls may be inadequate especially in the rural areas.

A Search for a Better Animal Model: Pigs may prove to be a superior model over mice for investigating immune modulation by dietary fish oil (i.e., omega-3 fatty acids)

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The primary objectives of this study were: (1) to explore the dose-response relationship between dietary omega-3 and immune cell fatty acid profiles in domestic pigs; (2) to compare the immune modulating activities of omega-3 fatty acids in pigs to those reported in mice and humans. Twenty male pigs (Landrace-Duroc cross) ~ 28 days old were housed in individual pens in a temperature-controlled environment. Pigs were randomly assigned to one of four dietary treatments (i.e., 0, 0.5, 1, 2% menhaden fish oil). At the end of a four-week feeding period immune cells from the blood and lungs were collected from each pig. Peripheral blood mononuclear cells (PBMC) and lung immune cells (mostly macrophages) were isolated. Immune cell and plasma phospholipid fatty acid analyses were carried out by standard gas chromatography. Ex vivo cytokine production of immune cells was stimulated with lipopolysaccharide (LPS) (0.1 ug/mL). The cell-free supernatant was collected after 24 h and [IL-1β] was quantified by ELISA. Our data suggest that the pig response to diet-induced changes in immune cell AA and DHA content matches that of humans better than that of the mouse. Pig immune cells appear to accumulate EPA to a much greater extent than human immune cells and thus this response is more similar to that found in mice. Pro-inflammatory cytokine production by porcine immune cells is reduced by dietary omega-3 enrichment.

Docosahexaenoic acid (DHA) protection in cortical neuron toxicity: Induction of 18-HEPE biosynthesis as an alternative pathway to protection

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Docosahexaenoic acid (DHA) is an omega-3 fatty acid enriched in the nervous system, which accumulated in cellular membranes phospholipids and enhances plasma membrane fluidity contributing to neuronal synaptic function. DHA is also liberated from cellular membranes and oxygenated to bioactive lipids via the action of 15-lipoxygenase type I initiated pathways that include, D-series Resolvins and Protectins which are potent modulators of inflammation and neuroprotection. For example, DHA and Neuroprotectin D1 protect from Aβ peptide-induced death in human neural cells and up-regulate the anti-apoptotic protein Bcl-2. Here we investigate the neuroprotective activity of DHA in glutamate-induced neuron cytotoxicity and the DHA-derived lipid products that might be formed in cortical neurons in vitro. We show that neurons increase their DHA-membrane by 5-fold after supplementation with 5μM DHA, increasing its neuron viability and preventing glutamate-induced toxicity. Protection was correlated with induction of Bcl-2 as well as in SOD2 and IDE mRNA and protein levels, target genes of the nuclear receptor PPARg. Employing LC/MSMS-based lipidomics we determined the formation of DHA-derived lipid mediators in cortical neurons exposed to DHA supplementation. Biosynthesis of NPD1 and resolvins was not observed in cortical neurons culture in vitro suggesting that the biosynthetic pathway of DHA-protective lipid mediators is not active. Of interest, we identified the production of 18-Hydroxyeicosapentaenoic acid (18-HEPE) which is known to be produced from EPA and is a precursor to RvE1. 18-HEPE was increased up to 10 fold in neurons treated with DHA and its addition to the cortical neuron cultures induced resistance against glutamate-induced damage. Together, our results show that DHA signaling is turn-off in rat cortical neuron cultures in vitro and instead an EPA-dependent pathway appears to be in place.

N-3 and N-6 Polyunsaturated Fatty Acids Have an Impact on Macrophage Respiratory Burst Against Rhodococcus Equi and Pseudomonas Aeruginosa

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Introduction: Macrophage oxidative metabolism is crucial for the innate immune response. However, some pathogens as R. equi and P. aeruginosa are able to evade these defense mechanisms leading to

chronic infections. Polyunsaturated fatty acids (PUFA) are known to modulate various immune response mechanisms including the production of reactive oxygen and nitrogen species (ROS/RNS). However, the impact of the fatty acid family, the carbon chain length and the unsaturation degree on the immunomodulating activity of PUFA is unknown so far. Here we present the first systematic study comparing the effects of various PUFA of both the n-3 and the n-6 family on macrophage respiratory burst.

Methods: RAW264.7 macrophages were supplemented for 72h with 15µM alpha-linolenic acid (LNA, C18:3n3), eicosapentaenoic acid (EPA, C20:5n3), docosahexaenoic acid (DHA, C22:6n3), linoleic acid (LA, C18:6n3) and arachidonic acid (AA, C20:4n6) respectively, and stimulated for 45min/6h/24h with phorbol-12-myristate-13-acetate, lipopolysaccharide as well as viable R. equi and P. aeruginosa. ROS and NO production were detected by dihydrorhodamine 123 and Griess reagent respectively (N=6, n=3, p<0.05).

Results: PUFA enrichment of unstimulated RAW264.7 was attended by a significant increase of ROS production depending on the unsaturation degree of the fatty acid supplemented and the supplementation duration. In contrast, for stimulated macrophages, PUFA enrichment at all time points resulted in a significant repressive effect on ROS synthesis. DHA was identified to be most effective. NO production was not affected by PUFA supplementation.

Conclusion: Our results underline the modulating effect of PUFA supplementation on macrophage respiratory burst against R. equi and P. aeruginosa. The analysis of ROS production demonstrates that long-chain PUFA, regardless of the fatty acid family, drive macrophage immune response into an anti-inflammatory direction. Thereby PUFA with a higher unsaturation degree are more effective in repressing the ROS synthesis than fatty acids with a lower unsaturation degree.

Green kiwifruit: effects on plasma lipids and APOE interactions

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Background: Diet is a crucial element in the reduction of risk of cardiovascular disease (CVD). Furthermore, response to dietary change may be influenced by genotype. Kiwifruit are a good source of several dietary components shown to improve dyslipidaemia and lower CVD incidence such as soluble fibre and some vitamins and phytochemicals.

Objective: To investigate the effect of consuming two green kiwifruit daily in conjunction with a healthy diet on plasma lipids and examine response according to apolipoprotein E (APOE) genotype in hypercholesterolaemic men.

Design: Eighty-five hypercholesterolaemic men (low-density lipoprotein cholesterol (LDL-C) >3.0 mmol/L and triglycerides (TG) <3 mmol/L) completed an eight week randomised controlled cross-over study, after undergoing a four week healthy diet phase. The study consisted of two 4-week treatment sequences of 2 green kiwifruit/day plus healthy diet (intervention) or healthy diet alone (control). Fasting blood samples were taken at baseline, 4 and 8 weeks for the measurement of plasma lipids (total cholesterol (TC), LDL-C, TG, high-density lipoprotein cholesterol (HDL-C)), serum apolipoproteins A1 and B (apoA1 and apoB).

Outcomes: After the kiwifruit intervention plasma HDL-C concentrations were significantly higher (mean difference 0.04 [95% CI: 0.01, 0.07] mmol/L [P=0.004]) and the TC/HDL ratio was significantly lower (-0.15 [-0.24, -0.05] mmol/L [P=0.002]), compared to control. In carriers of the APOE4 allele, TG concentrations were significantly lower (-0.18 [-0.34, -0.02] mmol/L [P=0.03]) after the kiwifruit intervention compared to control. There were no significant differences between the two treatments for plasma TC, TG, LDL-C and serum apoA1 or apoB.

Conclusion: The small but significant increase in HDL-C and decrease in TC/HDL-C ratio and TG (in APOE4 carriers) suggests that the regular inclusion of green kiwifruit as part of a healthy diet may be beneficial in improving the lipid profiles of men with high cholesterol.

Trial registration: ACTRN12610000213044

Adrenal Gland Fatty Acid Composition and Steroid Hormone Biosynthesis in Cyclic Ewes is Modified by Alpha-Linolenic Acid (ALA) Rich Diet

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Background: Experimental animal and human epidemiological and intervention studies indicate high consumption of n-3 polyunsaturated fatty acids (PUFAs) is associated with a reduced risk of cardiovascular and inflammatory bowel diseases and type 2 diabetes. In many Developed Western Countries, people consume foods that are low in n-3 and high n-6 PUFAs. Hence, there is a recognition of the need to increase n-3 PUFA content of foods in order to help ameliorate the problem. However, there is some concern as n-3 PUFAs have reported to influence biosynthesis of certain hormones.

Objective: To investigate the impact of ALA rich diet on steroid synthesis and adrenal PUFA composition in ewes.

Procedure: Two groups of Welsh Mountain ewes were fed either a control diet (n=8) or a diet supplemented with linseed high in ALA (n=8) for six weeks. The ewes were then culled and adrenal glands removed and used for isolation of adrenal cells or for fatty acid analysis. The isolated cells were cultured for 24h in serum-supplemented media. Spent media was analysed for cortisol by radioimmunoassay.

Results: Cortisol secretion was significantly higher in the ALA-rich diet fed ewe than in the control group (10.5 ± 1.8 ng/5×10^4 cells vs 6.4 ± 1.1 ng/5×10^4 cells, $p<0.05$). In adrenal gland, the ALA-rich diet group compared with their control counterparts had higher proportions of ALA (0.87 ± 0.10 vs 0.29 ± 0.11, $p<0.0001$), EPA (1.05 ± 0.33 vs 0.25 ± 0.10, $p<0.0005$), n-3 DPA (2.25 ± 0.46vs1.35 ± 0.34, $p<0.001$) and lower adrenic (0.92 ± 0.10 vs1.99 ± 0.66, $p<0.005$) and osbond (0.31 ± 0.06vs 0.59 ± 0.19, $p<0.005$) acids. The ALA-rich diet and control groups had comparable levels of linoleic (6.64 ± 1.37vs6.77 ± 1.16, $p>0.05$) and arachidonic (15.54 ± 1.86 vs16.70 ± 2.81, $p>0.05$).

Conclusion: n-3 fatty acid supplementation enhances biosynthesis of corticosteroids in Adrenal gland. There is evidence that ALA is used in de novo synthesis of cholesterol. Hence, the observed effect is likely to be due to the increased synthesis of cholesterol from ALA.

Atheroprotective effects and peroxidation of docosahexaenoic acid: is there a direct link?

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Docosahexaenoic acid (DHA), a long chain n-3 PUFA, is recognized for its cardioprotective effects with limitation of atherosclerosis progression. However, the 3 skipped dienes make this PUFA among the most susceptible to peroxidation in vivo, especially within the pro-oxidant environment of atherosclerotic plaque. Considering the paradox between DHA benefits and its susceptibility to peroxidation, we hypothesized that its peroxidized metabolites could contribute to the anti-atherogenic properties. The aim of the present study was first to analyze the relations between DHA peroxidation and atherosclerosis development and second to investigate the molecular mechanisms activated at the gene expression level. Transgenic LDLR-/- mice (n=30/group) were fed for 20 weeks a diet enriched with animal fat (10%, w/w) and cholesterol (0.045%, w/w) in paralell with daily oral gavages (5 days/week) with either oleic acid rich oil (Control) or a mixture of oils providing 0.1, 1 or 2% of energy as DHA (Group-1, -2, and -3, respectively). The preliminary results show that compared to Control, the highest dose of DHA reduced the systolic blood pressure (-16 mmHg, $p<0.01$), the levels of plasma cholesterol (-28%, $p<0.001$), triglycerides (-37%, $p<0.01$) and the extent of atherosclerotic plaque (-35%, $p<0.001$). Mass spectrometry was used to quantify peroxidized metabolites originating from n-6 and n-3 PUFA, namely 4-hydroxynonenal and 4-hydroxyhexenal-protein (HHE-P) adducts in liver as well as many oxylipins in plasma. The first collected data show an accumulation of DHA in liver (x8, $p<0.001$) and plasma (x2, $p<0.001$) of mice from the Group-3 compared to Control, in parallel with increased levels of liver HHE-P (+59%, $p<0.05$) and plasma DHA oxylipins (x3, $p<0.001$). Transcriptomic data from aorta samples are currently analyzed to provide information regarding the mechanisms of anti-atherogenic action of DHA and its metabolites. All together, these results show that DHA exerts atheroprotective effects despite the production of peroxidized molecules.

Effects of feeding different sources of omega 3 polyunsaturated fatty acids on liver, internal fat and muscle fatty acid composition in the lactating ewe

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Increasing the amount of omega-3 (n-3) polyunsaturated fatty acids (PUFA) in the tissues of agricultural species has potential for improving the health of consumers. The objective of this research was to determine changes in tissue fatty acid composition resulting from feeding n-3 PUFA from contrasting sources. Thirty-six lactating Rideau Arcott ewes were assigned randomly to one of five supplements (20g/d) for three weeks: a microalgae containing DHA (MA1), a microalgae containing EPA (MA2), flaxseed oil (FSO), an EPA/DHA enriched fish oil (FO) and hydrogenated cottonseed oil (CSO). Fatty acid analysis was conducted on internal fat, muscle and liver samples. There were no significant differences in the fatty acid profiles of each tissue when the CSO, FSO and MA2 treatments were compared. In liver tissue FO increased ($P<0.001$) the content of EPA (9.24%) compared with FSO (2.77%) and MA1 (1.68%). Both FO (11.75%) and MA1 (6.49%) supplements increased ($P<0.001$) the content of DHA compared with the FSO (2.4%). In muscle tissue the content of EPA (0.87%) and DHA (0.47%) were increased ($P<0.001$) by FO compared with FSO (0.23% and 0.02% respectively) and MA1 (0.34% and 0.19% respectively). In internal fat tissue EPA content was increased ($P<0.001$) by FO (0.13%) compared with FSO (0.03%) and MA1 (0.03%). The FO treatment was more effective in increasing the DHA content of the tissues even though there were approximately equivalent amounts of DHA in the MA1 supplement. Also, EPA and DHA were preferentially incorporated into liver tissues. Levels of the omega n-6 fatty acids linoleic (LA) and arachidonic (AA) varied with the lipid supplement but in contrast to the n-3 PUFA, tissue type affected the relative levels and direct correspondence with the supplement fatty acid profile was not always observed.

Effects of feeding different sources of omega 3 polyunsaturated fatty acids on gene expression of key enzymes and molecular regulators of lipid and carbohydrate metabolism in the liver of lactating ewe

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Feeding the lactating ewe long chain omega 3 polyunsaturated fatty acids (n-3 PUFA) enriches the DHA content of milk, however effects on lipid metabolism in the ewe need to be evaluated. The objective of this study was to determine the effects of feeding n-3 PUFA on expression of genes involved in lipid metabolism in the liver. Thirty-six lactating Rideau Arcott ewes were randomly assigned to one of five dietary supplements which included, a microalgae containing DHA (MA1), a microalgae containing EPA (MA2), flaxseed oil (FSO), an EPA/DHA enriched fish oil (FO) and hydrogenated cottonseed oil (CSO). The level of gene expression was determined for liver samples collected after feeding 20g/d of the supplement for a three week period. Expression of genes involved in liver de novo fatty acid synthesis (ACC, FAS), desaturation ($\Delta5$, $\Delta6$ and $\Delta9$ desaturase (D)) and transcriptional regulation (SREBP and ChREBP) was determined using qPCR. The expression of FAS decreased 53%, 56% and 67% with the FO supplement in comparison with the FSO, MA1 and MA2 supplements, respectively ($P<0.001$). The expression of $\Delta6D$ also decreased 50% and 71% with FO compared to MA1 and MA2, respectively ($P<0.001$). The expression of $\Delta6D$ decreased 35% with MA1 in comparison to FSO. ($P<0.001$). The expression of $\Delta9D$ decreased 68%, 50% and 71% with FO supplementation versus FSO, MA1 and MA2, respectively ($P<0.001$). The expression of SREBP decreased 10% with the FO supplement in comparison to the FSO supplement ($P<0.001$). The expression of ChREBP increased10% with the MA1 supplement in comparison to FSO ($P<0.001$). The dietary supplements had no significant effects on ACC and $\Delta5D$ expression. Significant effects on regulation of lipid metabolism were observed in this experiment which requires further investigation for determining the longer term impact on sheep production.

Does maternal supplementation with DHA during pregnancy enhance child development of attention or working memory and inhibitory control at 2 years of age: results of a randomised control trial

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Background: Docosahexaenoic acid (DHA) is an omega-3 fatty acid that accumulates in the fetal brain in the second half of pregnancy.

Objective: The aim of this study is to examine the effect of maternal supplementation with fish oil (a rich source of DHA) during the second half of pregnancy on attention, working memory and inhibitory control in childhood. Such functions reflect higher-order cognitive abilities known as Executive Functions (EF) and are dependent on the frontal lobes and hippocampus.

Methods: Children whose mothers were enrolled in the DOMInO trial (DHA to Optimise Mother and Infant Outcomes) were follow-up in a nested study. The DOMInO trial is a double-blind, randomized controlled trial in which pregnant women were randomly assigned to consume capsules containing 800mg/d of DHA (treatment) or vegetable oil (control) supplement from ~20 weeks until birth. The development of the frontal lobes and hippocampus was measured at 2 years of age using three assessments of attention and one of WMIC in a subset of DOMInO children who were born >37 weeks gestation and >2.5 kg. The primary outcomes were average latency for a child to be distracted when attention is focused (attention) and accuracy of finding a hidden toy during testing trials (WMIC).

Results: Assessments were completed by n=81 treatment and n=77 control group children. The primary outcomes for both assessments showed no effect of supplementation. There were no differences between the groups on all secondary outcomes except for one comparison involving multiple toys that provide competition for attention where the intervention group children looked away from the target stimuli fewer times than controls (treatment group: mean=13.95 (SD=5.75).

Conclusion: Further investigation is necessary to establish whether there is a clear association between DHA supplementation during pregnancy and EF in childhood.

Long Chain Omega-3 Polyunsaturated Fatty Acids: Effects on Plasma Adipokine Levels in Obese, Non-Diabetic Humans

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Alterations in the circulating concentrations of the adipokines leptin and adiponectin have been implicated in the aetiology of insulin resistance, hypertension and dyslipidemia, all of which are risk factors for heart disease progression. Animal studies have provided evidence that increased intake of omega-3 long chain polyunsaturated fatty acids (LCPUFA) is associated with a more metabolically favourable adipokine profile, including increased adiponectin levels and reduced leptin levels. This study aimed to determine the effects of omega-3 supplementation in overweight/obese individuals.

Forty non-diabetic participants (10 overweight, 30 obese) were enrolled in the study. Each participant was supplemented with 4g/day of a commercially available marine lipid preparation (equivalent to 2g/day EPA/DHA). Fasting blood samples were taken at baseline, and following 4 and 8 weeks of supplementation. We assessed changes in plasma adipokine levels (adiponectin, leptin and acylated ghrelin), along with markers of insulin sensitivity, blood lipids and liver function. Results were correlated with changes in plasma fatty acid phospholipid profiles. Validated physical activity (IPAQ), fatigue (FIS) and dietary intake questionnaires were also completed at each of these intervals.

Contrary to previously published results in animal studies, omega-3 LCPUFA supplementation had no significant effect on adipose hormones in these individuals. Results did, however, suggest a number of other benefits associated with omega-3 LCPUFA supplementation including decreased levels of physical, social and mental fatigue. Obesity associated increases in fatigue are thought to play an integral role in poor weight management, restricting physical activity levels. Given these preliminary results, further quantitative studies are recommended regarding the effects of omega-3 LCPUFA on obesity related fatigue and the subsequent impact on quality of life, health-care and weight loss interventions.

Elongase reactions as control points in long-chain polyunsaturated fatty acid synthesis

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Background - Metabolism of α-linolenic acid (18:3n-3; ALA) to eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA), requires progressive desaturation and elongation. Δ6-Desaturase (Fads2) is regarded as rate-limiting in the conversion of ALA to DHA. However, increasing the direct Fads2 product stearidonic acid (18:4n-3; SDA) increases tissue levels of EPA and docosapentaenoic acid (22:5n-3; DPA), but not DHA. This suggests that other control points need to be considered. The accumulation of EPA and DPA merit a systematic examination of the elongase enzymes involved in their metabolism. One possible control point is the second reaction involving Fads2, 24:5n-3→24:6n-3.

Objective - Examine the activities of the rat elongase enzymes, as well as the second reaction of Fads2, to better understand the metabolism of EPA to DHA.

Methods - The rat Elovl2, Elovl5 and Fads2 sequences were cloned and the enzymes expressed in Saccharomyces cerevisiae. Recombinant S. cerevisiae cells were cultured in the presence of various C18, C20 and C22 polyunsaturated fatty acid (PUFA) substrates to determine the substrate specificity of the enzymes. Competitive substrate interactions and dose response curves were examined.

Results - Rat Elovl2 was active with C20 and C22 PUFA and this single enzyme catalysed the sequential elongation reactions of EPA→DPA→24:5n-3. The second reaction DPA→24:5n-3 appeared to be saturated at substrate concentrations not saturating for the first reaction EPA→DPA. ALA dose-dependently inhibited Fads2 conversion of 24:5n-3 to 24:6n-3.

Conclusion - The competition between ALA and 24:5n-3 for Fads2 may explain the decrease in DHA levels observed after certain intakes of dietary ALA have been exceeded. In addition, the apparent saturation of the second Elovl2 reaction, DPA→24:5n-3, provides an additional explanation for the accumulation of DPA but not DHA when dietary ALA, SDA or EPA is increased. Our findings suggest that Elovl2 will be critical in understanding if DHA synthesis can be increased by dietary means.

Docosahexaenoic acid supplementation during pregnancy results in higher fetal heart rate variability at 36 weeks gestation

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Background: Prenatal DHA supplementation has been previously reported to affect fetal cardiac autonomic control in a small observational study.

Objective: This study sought to investigate the effect of maternal DHA supplementation on fetal heart rate (HR) and HR variability (HRV) in a randomized clinical trial.

Methods: Women were randomly assigned to three capsules containing a total of 600 mg DHA (n=22) or placebo (soybean-corn oil) (n=24) beginning at 12-20 wks gestation. Blood was obtained at enrollment and delivery (maternal and cord); red blood cells (RBCs) were isolated, extracted, and phospholipid (PL) isolated, fatty acids derivatized to methyl esters (BF3-methanol) and quantified (wt% of total fatty acids) by gas liquid chromatography. Two 18 minute magnetocardiograms were recorded at 36-wks gestation. Fetal HR and HRV metrics were subjected to an intent-to-treat analysis using a mixed-effects model.

Results: RBC-PL DHA in the groups did not differ at enrollment [DHA, 5.0% ± 1.4%; Placebo, 4.7% ± 1.2%]. Supplementation had a significant effect on maternal [DHA, 7.5% ±2.5%; Placebo, 5.2% ±0.9% (p=0.002)] and infant RBC-PL DHA [DHA, 7.8% ±2.0%; Placebo, 6.4% ±1.1% (p=0.035)] at delivery. In the supplemented group, fetal HR was lower (but not significantly so; p=0.20) and time-domain measures of fetal HRV were significantly higher: Overall HRV (LogSDNN, p=0.035), short-term HRV (Log RMSSD p=0.051). Frequency-domain measures were significantly higher in all frequency bands (Log VLF, p=0.025; Log LF, p=0.036 and log HF p=0.054).

Conclusion: Maternal DHA supplementation during the 2nd and 3rd trimesters of pregnancy significantly affected fetal cardiac autonomic function as indexed by HRV metrics influenced by both sympathetic and

parasympathetic activity. These results are consistent with the possibility that DHA has a programming effect that may contribute to advantageous or adaptive cardiac autonomic function.

Reversal of fatty liver and hepatocellular injury by a docosahexaenoate ethyl ester and N-acetylcysteine in rats fed a high fat diet

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This study compared the efficacy of: 1) a highly concentrated ethyl ester of docosahexaenoic acid (MATK-90, 900mg/g), 2) N-acetylcysteine (NAC), and 3) MATK-90+NAC to reverse hepatocellular injury resulting from diet-induced non-alcoholic steatohepatitis (NASH). Effects of treatment were compared using NASH activity scores (NAS) based on histology and plasma biochemistry. Male rats (250-300 g) were fed a low fat diet (LFD) with 16.4% of total kJ from soybean oil (7%/g diet), or a high fat diet (HFD, 58% of total kJ from fat) containing 33.35% and 0.25%/g diet of oil from coconut and soybean, respectively. MATK-90 (2 g/kg) and NAC (0.6 g/kg) were administered by oral gavage daily. At 150 days, after NASH induction, the HFD-fed animals were allocated to three new treatment groups: HFD+MATK-90, HFD+NAC, or HFD+MATK-90+NAC for 30 additional days. Untreated-HFD and LFD groups were also followed for 30 days.

The NAS classifications included: not present (0), possible (1), and definite (2) and were based upon the presence of hepatocellular steatosis, ballooning, and lobular inflammation. At 150 days, the NAS for the LFD and HFD groups were 1.8+/-0.8 and 2.7+/-1.2, respectively, with inflammation in 3/6 HFD-fed animals. HFD treatment increased liver TG concentrations by 57% ($p<0.01$); levels of alanine (ALT) and aspartate (AST) aminotransferase increased significantly ($p<0.02$) compared with animals fed the LFD diet.

After 30 days of treatment (days 150-180), both MATK-90+NAC and MATK-90 were effective in reducing NAS scores (~40%, $p<0.05$), steatosis (50%, $p<0.05$) and ballooning (80%, $p<0.05$). Only MATK-90+NAC decreased hepatocellular lipid storage by 50%-60% ($p<0.05$) and the levels of ALT ($p<0.03$) and AST ($p<0.07$) by 43% relative to the untreated-HFD and LFD groups. The results indicated that the combination of MATK-90+NAC effectively mitigated and reversed key features of NASH in rats.

Fatty acid composition in the postmortem entorhinal cortex of patients with schizophrenia, bipolar disorder and major depression

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Background: We have previously investigated n-3 long-chain polyunsaturated fatty acids (LCPUFAs) in the post-mortem hippocampus from subjects with schizophrenia and bipolar disorder, and controls; however, we found no significant differences except for small ones in n-6 LCPUFAs (Hamazaki et al. J Psychiatr Res 2010). The post-mortem amygdalae showed no significant differences in major LCPUFAs, either (Hamazaki et al. in submission). Other studies with post-mortem orbitofrontal cortex showed abnormalities in n-3 LCPUFAs in individuals with schizophrenia, major depression and bipolar disorder (McNamara 2007a, 2007b, 2008). In the present study we investigated whether there were any abnormalities in LCPUFAs in the entorhinal cortex of schizophrenia, bipolar disorder and major depression compared to unaffected controls.

Methods: We obtained from the Stanley Medical Research Institute 15 entorhinal cortex samples each for schizophrenia, bipolar disorder, major depression and controls matched for age, gender, race, post-mortem interval, brain pH and laterality of hemisphere. Entorhinal cortex tissues were scraped off from 3 consecutive frozen sections for microscopic slides (14 μm each) and homogenized. Total lipids were extracted and total phospholipid fractions were separated by thin-layer chromatography. The fatty acid composition was analyzed by gas chromatography.

Results: Unlike the previous studies with the orbitofrontal cortex and hippocampus, we found no significant differences in major LCPUFAs. The amounts of docosahexaenoic acid (%), the major n-3 LCPUFA, were 11.2 ± 1.5, 11.8 ± 0.9, 11.1 ± 1.4 and 11.9 ± 0.9 in schizophrenia, bipolar disorder, major

depression and unaffected controls, respectively. The composition of arachidonic acid (%), the major n-6 LCPUFA, was 9.1 ± 1.2, 9.7 ± 0.8, 9.7 ± 0.5 and 9.9 ± 0.6, respectively in the same order.

Discussion: Changes in LCPUFAs in these psychiatric disorders may be specific to certain brain regions. LCPUFAs in the entorhinal cortex may not be the etiology of these diseases.

Fatty acid composition and estimated desaturase activities in serum lipids of obese children with and without multiple cardiovascular risk factors

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The aim of this study was to identify the relationship between serum fatty acid (FA) composition, various desaturase indices and the co-morbidity of metabolic syndrome in obese children.

Methods: Thirty-eight obese children (23 male, 15 female) aged 10.2 ± 2.4 years (mean ± SD) were recruited. Serum FA composition was determined by gas chromatography. Desaturase indices were calculated form product/substrate ratio. Metabolic syndrome (MetS) was defined using Japanese MetS criteria for children. Using the number of cardiovascular risk factors (RFs) the subject divided into two groups (non-MetS group: no. of RF s = 0 or 1, MetS-like group: no.of RFs more than 2).

Results: No sex difference was found in FA composition or desaturase activity indices. We found significant positive correlations between waist circumference (WC), serum TG levels, number of RFs and volume% of MUFA (r = 0.423, 0.813, 0.642, p<0.01). There were inverse correlations between serum TG, number of RFs and serum n-6FUFA (r = -0.807, -0.679, p< 0.01). In addition, SCD indices and D6D had significant positive relationship with all of MetS co-morbidities except high fasting blood glucose. D5D correlated inversely with all components of MetS. MetS-like children exhibited significance higher levels of SFA, MUFA, SCD16, SCD18 than non-MetS children. (31.3±2.0 vs. 30.0±1.0, 24.9±1.3 vs. 20.9±1.3, 0.11±0.02 vs. 0.09±0.01, 2.9±0.5 vs. 2.5±0.3, 0.06±0.02 vs. 0.05±0.01, p<0.05). In contrast n-6PUFA and D5D values were significantly lower in MetS-like group than non-MetS group (33.2±3.5 vs. 37.7±1.8, 3.8±1.1 vs. 5.1±1.7, p<0.01).

Conclusions: There was significant relationship between serum FA composition and co-morbidities of childhood MetS. Increased serum MUFA and reduced n-6PUFA levels associated with deranged desaturase activities may contribute to development childhood MetS.

Dietary n-3 Fatty Acid Deficiency Enhances Anxiety Induced by Chronic Mild Stress in Mice

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Docosahexaenoic acid (DHA), is the major polyunsaturated fatty acid in the brain and is important for both the structure and the function of the nervous system. The aim of this work is to measure the anxiety level using the dietary n-3 fatty acid deficient mice. Mice were fed either an n-3 fatty acid deficient (n-3 Def) or adequate (n-3 Adq) diet for two generations. Also the mice were housed under two conditions, as a group or in isolation and the major point of the study was to determine whether n-3 fatty acid deficiency would enhance isolation induced anxiety. Isolation stress was assessed using the novelty suppressed feeding paradigm (NSF) after a 3-week period and the test lasted a maximal duration of 10 min. The number of successful mice consuming food pellets within 5 min in the n-3 Def diet group was low in both housing conditions (group housing, 33% and isolated, 30%), but was 92% in the group housed and 50% in the isolated group when fed the n-3 Adq diet. In the subsequent 5 min period, the isolated housing group consuming the n-3 Adq diet increased up to 79% and the group housed animals fed the n-3 Def diet increased to 67%. However, those that consumed the n-3 deficient diet combined with isolation stress exhibited no increase. These results suggested that the n-3 deficient mice had increased anxiety that was enhanced by the chronic mild stress of social isolation.

Red blood cell fatty acid levels improve GRACE score prediction of 2-yr mortality in patients with myocardial infarction

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Background: Blood omega-3 and omega-6 fatty acid levels have been associated with reduced risk for total mortality in patients with stable coronary heart disease (CHD), but their relationships with mortality in the setting of myocardial infarction (MI) are unknown.

Objective: To determine the association between red blood cell (RBC) fatty acid levels measured at admission and 2-year mortality in MI patients, independent of the GRACE risk score, a traditional mode of risk stratification,

Design: Admission RBC fatty acid levels were measured in patients enrolled in a prospective, 24-center MI registry (TRIUMPH). Two-year mortality was modeled with Cox proportional hazards regression to assess the extent to which the inclusion of fatty acid levels would improve, over and above the GRACE score, risk stratification for 2-year mortality.

Results: RBC fatty acid data were available from 1,144 patients who did not report taking fish oil supplements after discharge. Two RBC fatty acids [eicosapentaenoic acid (EPA n-3) and docosapentaenoic n-6 (DPA)] were univariate predictors of total mortality. The combined fatty acid c-statistic (0.60, $p<0.001$) improved the c-statistic of the GRACE score alone from 0.747 ($p<0.001$) to 0.768 ($p<0.05$ vs. GRACE alone). The net reclassification index improved by 31% (95% CI, 15%,48%) and the relative incremental discrimination index improved by 19.8% (7.5% to 35.7%).

Conclusion: RBC EPA and DPA n-6 levels improved the prediction of 2-yr mortality over and above the GRACE score in MI patients.

Red Blood Cell Omega-3 Fatty Acid Levels and Markers of Accelerated Brain Aging: The Framingham Heart Study

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Background: Higher dietary intake and circulating levels of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have been related to a reduced risk for dementia, but the pathways underlying this association remain unclear.

Objective: To examine the cross-sectional relation of red blood cell (RBC) fatty acid levels to subclinical imaging and cognitive markers of dementia risk in a middle-aged to elderly community-based cohort.

Methods: We related RBC DHA and EPA levels in dementia-free Framingham Study participants (N= 1,575; 854 women, age 67±9 years) to performance on cognitive tests and to volumetric brain MRI, with serial adjustments for age, sex and education (Model A, primary model), additionally for ApoE ε4 and plasma homocysteine (Model B), and also for physical activity and body mass index (Model C), or for traditional vascular risk factors (Model D).

Results: Participants with RBC DHA levels in the lowest quartile (Q1) when compared to others (Q2-4) had lower total brain ($p=0.009$) and greater white matter hyperintensity volumes ($p=0.049$), with persistence of the association with total brain volume in multivariable analyses. Participants with lower DHA and omega-3 index (RBC DHA+EPA) levels (Q1 versus Q2-4) also had lower scores on tests of visual memory ($p=0.008$), executive function ($p=0.004$) and abstract thinking ($p=0.004$) in Model A, the results remaining significant in all models.

Conclusion: Lower RBC DHA levels are associated with smaller brain volumes and a 'vascular' pattern of cognitive impairment even in persons free of clinical dementia.

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Effects of fish oil on blood pressure and plasma lipid profile in a randomized controlled intervention in healthy Danish infants

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Background: Our previous uncontrolled trial showed that supplementation with marine n-3 fatty acids (n-3PUFA) reduced blood pressure (BP) and plasma triacylglycerols (TAG) and increased HDL-cholesterol in healthy 9-month-old infants.

Aim: To explore if these effects are caused specifically by n-3PUFA we performed a new trial, where 9-month-old infants received fish oil (FO) or sunflower oil (SO) for 9 months (5 mL/day).

Methods: Diastolic and systolic BP (dBP & sBP), plasma TAG, total and HDL-cholesterol, and erythrocyte (RBC) fatty acid (FA) composition were measured before and after the intervention after a mean fasting time of 168±128 and 157±48 min, respectively. 133 (86% of the recruited) infants completed the intervention and BP was measured at both 9 and 18 months in 109, of whom 106 also had RBC-FA data.

Results: Total PUFA-intake at 18 months was 5.4 and 6.5E% hereof ~2E% and 0.7E% n-3PUFA in the FO- and SO-group, respectively, resulting in a ~2-fold increase in RBC n-3PUFA in the FO-group. We were unable to measure BP at 18 month in 7% and 18% of the boys and girls in the FO-group versus 25% and 19% in the SO-group (Chi2 p=0.065 for boys). In the rest BP increased during the intervention and at 18 month mean (95%CI) sBP/dBP adjusted for 9-month-BP and gender was 113 (109;116)/68 (65;71) mmHg in the FO-group versus 116 (113;120)/67 (64;70) mmHg in the SO-group (p=0.203 for sBP). Plasma TAG increased from 9 to 18 month, but less in the FO-group (0.7 (0.5;1.0) versus 0.9 (0.6;1.3) mmol/L in the SO-group, p=0.002 adjusted for baseline and gender), but total cholesterol and HDL were not significantly affected by the intervention.

Conclusion: The results are in line with the results of our previous trial. The long-term consequences of the effects remain unknown, but might suggest a suboptimal n-3PUFA-status in early childhood.

Effects of long-term administration of arachidonic acid on spatial cognition in aged rats

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[Background] Arachidonic acid (20:4n-6, AA), one of main structural fatty acids of many tissues including neuronal tissues, plays an important role in neuronal functions such as learning and memory. The amount of AA in brain is lower in aged rats compared with young rats, associating with age-related cognitive deficits. It is, thus, suggested that the possibility that cognitive learning deficits of aged rats may be improved if aged rats were administered with AA, but there are few reports. We investigated whether long-term administration of AA affects cognitive learning ability in aged rats.

[Materials] Wister rats were provided with a fish oil-deficient diet. Inbred second-generation male aged rats (100 weeks old) were divided into two groups: the AA group (n=8), which was orally administered AA-enriched triacylglycerol (AA: 240 mg/kg/day) for 13 weeks; and the control group (n=7), which was orally administered the control oil (beef, soybean and palm-mixed oil) for 13 weeks.

[Results] Final body weights did not differ between the AA and control groups. Administration of AA did not affect the number of reference and working memory errors in an 8-arm radial maze, suggesting that long-term administration of AA does not improve age-related cognitive deficits in aged rats. However, the total time to get all reward pellets in the radial maze is shorter in AA-administered rats compared with control rats. Administration of AA significantly increased the levels of AA in plasma and cerebral cortex of aged rats, suggesting the AA cascade hypothesis in brain. The administration significantly increased the plasma levels of lipid peroxide, but did not affect the levels of lipid peroxide in the cerebral cortex and hippocampus of aged rats.

[Conclusion] Long-term administration of arachidonic acid to aged animals may be related with neuronal dysfunctions such as mood disorders.

The Effects of High Dose Fish Oil Supplementation During Infancy and FADS1 & 2 Genetic Polymorphisms on Neurocognitive Outcomes at 6 Years

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Introduction: Omega 3 (n-3) long-chain polyunsaturated fatty acids (LCPUFAs) accrete within the grey matter of the cerebral cortex during fetal and infant development, and are thought to modulate cerebral development. n-3 LCPUFAs can be obtained through the diet or synthesised from n-3 fatty acid precursors. The genes which regulate the production of LCPUFAs from fatty acid precursors are FADS1 and FADS2. It is proposed that dietary intake of n-3 LCPUFAs and their n-3 precursors, plus a specific genetic predisposition, modulate n-3 LCPUFA levels in humans and affect neurodevelopment.

Methods: In this randomised, double-blind, placebo-controlled trial, 420 healthy term infants were assigned to receive either n-3 LCPUFA supplementation in the form of fish oil (containing at least 250mg DHA and 60mg EPA per day) or a placebo (olive oil) from birth to six months. The present study undertook neurocognitive assessment on this cohort at age 6 in addition to genetic profiling for FADS1 and FADS2.

Aims & Hypothesis: To evaluate the effects of high-dose fish oil supplementation during infancy on neurodevelopmental outcomes during childhood. The two-way interaction between genetic profile and n-3 LCPUFA supplementation on child neurocognitive development was examined. We hypothesized i) that supplementation with fish oil during early infancy would result in significant enhancements of neurocognitive skills (relative to placebo), and ii) that examination of single nucleotide polymorphisms (SNPs) within FADS1 and FADS2 candidate genes would reveal a genetic subgroup that was better equipped to metabolise dietary n-3 LCPUFAs, and therefore receive greater neurocognitive benefit from n-3 LCPUFA supplementation.

Impact of n-3 supplementation on fatty acid composition of erythrocytes, plasma, and breast tissue in women at increased risk for breast cancer

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Background: In an observational study, we have found relationships between preneoplastic biomarkers in breast tissue and EPA and DHA content in phospholipid (PL) and triacylglycerol (TAG) fractions of erythrocytes and plasma. We hypothesized that EPA and DHA supplementation could reduce breast cancer risk biomarkers by increasing tissue n-3 PUFA; this study is ongoing. Here we report the effect of supplementation and withdrawal on PL and TAG composition of erythrocyte, plasma and breast.

Methods: Women (n=8 of proposed 60) at increased risk for breast cancer took LovazaTM (4 g/d; 1800 mg EPA and 1500 mg DHA) for 6 months in a single-arm study. We obtained blood at 0, 6, and 6.5 months and breast tissue before supplementation and at 6.5 months by random periareolar fine-needle aspiration. The fatty acid composition of erythrocytes, plasma, and breast tissue TAG and PL were analyzed by gas liquid chromatography and expressed as weight% of total fatty acids. Statistical analysis was performed using two-sided Wilcoxon signed rank test.

Results: Pre-study EPA, DHA, and AA in plasma TAG (0.20, 0.39, 1.71%, respectively) were about 4-fold higher than in breast TAG (0.04, 0.12, 0.39%). After 6 months of intervention with LovazaTM DHA content of erythrocyte PL increased from 3.0 to 5.4% (p=0.018), as did n-3:n-6 ratio of erythrocyte (0.19 to 0.49, p=0.018) and plasma (0.13 to 0.39, p=0.018) PL, and the n-3:n-6 ratio in breast (0.07 to 0.10, p=0.05) TAG. EPA+DHA:AA ratio increased in breast TAG (0.34 to 0.94, p=0.012).

Conclusion: LovazaTM increases the n-3 content in erythrocytes, plasma, and breast. Though breast tissue is much lower in long-chain PUFA compared to erythrocyte and plasma PL and TAG, supplementation increases n-3:n-6 and DHA+EPA:AA ratios in breast TAG.

Expression patterns of lipid metabolism associated genes and gene products indicate that liver and omentum are the key responders to a dietary fatty acid manipulation of lactating dairy cows

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Background: Elucidating cellular and systemic mechanisms regulating lipid metabolism pathways is of crucial interest to optimize health and performance parameters of farm animals, particularly during physiologically demanding phases.

Materials/Methods: German Holstein cows (n=18; ~90 DIM) were subjected to a rumen-protected saturated fat (n=6), sunflower oil/algae (n=6) or linseed oil/algae (n=6) supplemented diet. Hepatic, longissimus muscle and subcutaneous/perirenal/omental adipose tissue samples were taken after a 10-week feeding trial and subjected to expression analyses of lipid metabolism associated genes and gene products. Lipogenesis-related transcription factors and nuclear receptors (CEBPA/B, PPARG, SREBF1), lipogenic enzymes (ACACA, FASN, SCD, FADS1, FADS2), lipid storage proteins (ADFP), lipid trafficking proteins (CD36, LPL, MTTP) and carbohydrate/lipid metabolism bridging proteins (ACLY) were addressed.

Findings: The study revealed that a plant oil/algae intervention primarily shifted lipid metabolism gene expression levels in hepatic tissue and omental adipose tissue. In hepatic tissue, reduced expression of ACACA, FADS1, FADS2, FASN, SCD and SREBF1 gene was obtained, whereas in omental adipose tissue, up-regulated expression of ACACA, ADFP, CEBPA, FASN, LPL, PPARG, SCD and SREBF1 gene was found. Despite majorly shifted gene expression levels in hepatic and omental adipose tissue, gene/gene product correlations were found to be comparatively lower than in muscle, perirenal adipose and subcutaneous adipose tissue, indicating matches only in regard to minor concentrations of SCD product C18:1c9, FADS1 product C20:4n-6 and FADS2 product C18:3n-6 in hepatic tissue, and higher concentrations of ACACA and FASN gene products C12:0 and C14:0 and SCD product C18:2c9,t11 in omental adipose tissue.

Conclusion: Expression patterns of lipid metabolism associated genes and gene products were tissue-specially shifted upon a dietary fatty acid intervention, highlighting hepatic tissue and omental adipose tissue as the key responders to a dietary fatty acid manipulation, and outlining the central role of these tissues in the systemic lipid metabolism regulation of lactating dairy cows.

Dietary intervention with different PUFA affects lipid metabolism and lipid profile of different tissues of German Holstein bulls and cows

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The study investigated the long-term effect of different dietary PUFA on lipogenic enzymes and lipid profiles of different tissues of German Holstein bulls and cows.

In the first experiment, 29 German Holstein bulls were assigned to two treatment groups. The control group received a C18:2n-6 (soybean meal, maize silage) and the experimental group a C18:3n-3 (linseed oil, rapeseed cake, grass silage) supplemented diet. In the second experiment, 18 lactating Holstein cows were assigned to one of three feeding groups [saturated fat (SAT) (3.1% TMR DM), linseed oil (LINA) (2.7% TMR DM) or sunflower oil (SUNA) (2.7% TMR DM) added with DHA rich algae (0.4% TMR DM)] during a ten-week intervention.

In the first trial, finished bull muscle contents of C18:3n-3 and the sum of n-3 fatty acids were significantly higher in the experimental than in the control group, whereas the n-6/n-3 PUFA ratio was significantly lower in the experimental group. In agreement with lower MUFA concents, SCD protein expression and SCD activity was significantly minor in experimental than control group muscle tissue.

In the second trial, n-3 FA supplementation (LINA) of lactating cows caused significantly elevated n-3 PUFA contents in intramuscular fat and milk fat, whereas n-6 FA supplementation (SUNA) increased n-6 FA contents in muscle tissue and milk. Significantly reduced milk fat contents and saturated fatty acid amounts (C10:0, C12:0, C14:0, C16:0) were obtained in LINA and SUNA compared to SAT group. The

pSREBP-1 protein expression was in tendency reduced in SUNA group mammary gland tissue (P=0.087), whereas no effect on protein expression levels of transcriptionally active mSREBP-1 was obtained. Upon PUFA feeding, elevated trans FA contents were detected, in milk fat rather than in intramuscular fat.

To conclude, long-term feeding of exogenous PUFA results in an accumulation of these essential FA in different tissues of bulls and cows.

Effect of serum fatty acid binding protein 4 on anthropometrical and metabolic parameters and adipocyte fatty acid composition.

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Aim: Fatty acid-binding protein 4 (FABP4) belongs to the family of lipid chaperones expressed in adipocytes and bind free fatty acids during lipolysis. Some studies have shown that serum levels of FABP4 secreted from adipocytes is associated with obesity, insulin resistance, and atherosclerosis. Our study was focused on effect of FABP4 on anthropometrical and metabolic parameters and fatty acid composition in adpocytes.

Methods: We examined a group of 67 obese subjects (15 men, 52 women), BMI 34.1±4.6 (mean±SD). Anthropometric parameters were measured following standardized procedures. Parameters of lipid and glucose metabolism were assessed in fasting plasma samples. Serum FABP4 level was measured using a commercially available enzyme-linked immunosorbent assay kit. Fatty acid composition of subcutaneous adipose tissue was analyzed by gas chromatography.

Results: Serum FABP4 level positive correlated with BMI (p<0.001) and with percentage of fat mass (p<0.001). The correlation with percentage of fat mass remained significant after adjustment data for BMI (p<0.01). Correlations with other anthropometrical parameters (waist and hip circumferences, sagittal abdominal diameter, fat free mass) were not significant. No significant correlation was found between serum FABP4 levels and lipid (triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol) and glucose metabolism parameters (fasting glucose, insulin, C-peptide, HOMA-IR) after adjustment for BMI. Serum FABP4 level had no effect on composition of fatty acids in adipocytes from subcutaneous adipose tissue.

Conclusion: The results confirm association of serum FABP4 levels with obesity. Association with parameters of lipid metabolism and parameters of insulin resistance were not significant after adjustment for BMI, moreover other factors may play important role except for FABP4. Our data suggest no effect of serum FABP4 levels on fatty acid composition in adipocytes from subcutaneous adipose tissue.

Fat content of maternal diet alters both male and female offspring fatty acid status through epigenetic regulation of FADS2

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There is considerable evidence that nutrition during development, through altered epigenetic regulation of specific genes, induces persistent changes in offspring phenotype. Little is known about the effect of nutrition during development on future capacity for polyunsaturated fatty acid (PUFA) biosynthesis. We investigated the effect of feeding pregnant and lactating rats different amounts and types of fat on PUFA metabolism in adult offspring.

Dams were fed diets enriched in saturated fatty acids (butter), or 20:5n-3 and 22:6n-3 (fish oil, FO) at either 3.5%, 7% or 21%(w/w) from conception until offspring were weaned onto AIN93G containing 4%(w/w) soybean oil. Offspring livers were collected on day 77. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) PUFA compositions were measured by gas chromatography. mRNA expression of FADS2, which encodes Δ6 desaturase, was measured by real time RTPCR. Methylation of the FADS2 promoter was measured by pyrosequencing.

Male offspring of dams fed 21% fat had significantly (P<0.05) lower 20:4n-6, and female offspring had lower 20:4n-6 and 22:6n-3 in liver PC and PE than offspring of dams fed 3.5% or 7% fat irrespective of fat type. FADS2 mRNA was lower (P<0.005) in offspring of dams fed 21% fat compared to other groups. Methylation at the CpG dinucleotide at locus -394bp relative to the transcription start site, which is known to modify FADS2 transcription, was greater in offspring of dams fed 21% fat (P<0.001), irrespective of type of fat.

These data show that the amount of maternal fat can induce persistent changes in 20:4n-6 and 22:6n-3 status. Such changes in PUFA status involve altered epigenetic regulation of FADS2 by DNA methylation. Together these findings may have implications for regulation of membrane composition and cell function. One further implication is that such effects may affect the capacity of the female offspring when pregnant to provide sufficient PUFA to their developing offspring.

Combined effects of fish oil and taurine or soy protein on hyperglycemia and hypercholesterolemia in mice

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The long-chain n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are well known to have health beneficial effects such as anti-hyperlipidemia and anti-hyperglycemia activities. To utilize fish oil containing EPA and DHA on human health, combination with other functional compounds is considered to exhibit effective functions. In this study, we focused on taurine and beta-conglycinin as other functional compounds, because they have been reported to decrease serum cholesterol level and improve insulin resistance. To investigate combined effect of fish oil and taurine or beta-conglycinin, diabetic/obese KK-Ay mice and C57BL/5.KOR/StmSlc-Apoe (ApoE deficient), which are hyperglycemia and hypercholesterolemia model mice, were fed experimental diets containing fish oil and taurine or beta-conglycinin for 4 weeks. Combination of fish oil and taurine significantly suppressed white adipose tissue (WAT) weight gain of KK-Ay mice compared to taurine or fish oil diets. Furthermore, in fish oil + taurine group, hyperglycemia and insulin level were effectively improved compared to the fish oil only group. The combination of fish oil and taurine enhanced the glucose transporter 4 translocation in the plasma membrane of skeletal tissue in KK-Ay mice. On the other hand, combination of fish oil and beta-conglycinin markedly decreased cholesterol level in apoE deficient mice through down-regulation HMG-CoA reductase mRNA expression in the liver. These results suggest that EPA and DHA-rich fish oil and taurine or beta-conglycinin is the effective combination to prevent and improve diabetes, obesiy and hypercholesterolemia.

High plasma phospholipase A2 activity is associated with low levels of eicosapentaenoic acid and docosahexaenoic acid incorporation in erythrocytes following fatty acid supplementation

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Background - Phospholipase A2 (PLA2) enables functions of polyunsaturated fatty acids (PUFA) by releasing them from membrane stores. Thus abnormalities in PLA2 activity could account for the altered PUFA status observed in various health conditions, including mood, memory and cognitive decline associated with older age, and for individual variation in response to omega-3 (n-3) PUFA supplementation in this group.

Objective - To explore relationships between plasma PLA2 activity and increases in erythrocyte PUFA status in response to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) supplementation.

Design - Fifty adults ≥ 65 years with MCI were recruited for a 6-month double-blind placebo-controlled parallel trial. Volunteers were randomly allocated to consume an EPA-rich oil (1670 mg EPA + 160 mg DHA/day), DHA-rich oil (400 mg EPA + 1550 mg DHA/day) or linoleic acid-rich oil (safflower oil, 2200mg LA/day). Plasma PLA2 activity was assessed at baseline, erythrocyte PUFA status was assessed at baseline and six months.

Outcomes - 38 volunteers completed the trial. After supplementation with DHA for 6 months (n=15), lower baseline PLA2 activity was associated with larger increases in EPA (r=-.66, p=.007), n-3

docosapentaenoic acid (DPA, r=-.59, p=.021), DHA (r=-.79, p<.0001) and total n-3 PUFA (r=-.76, p=.001) and with smaller reductions in total omega-6 (n-6) PUFA (r= -.52, p=.050). Lower baseline PLA2 activity predicted greater increases in the n-3/n-6 ratio (r= -.75, p=.001). After supplementation with EPA for 6 months (n=12), lower baseline PLA2 activity was associated with larger increases in n-3 DPA (r=-.65, p=.021), DHA (r=-.62, p=.030) and total n-3 PUFA (r=-.66, p=.018) and smaller reductions in arachidonic acid (r=-.59, p=.04).

Conclusion - Variation in PLA2 activity may account for some of the observed differences between individuals in the incorporation of n-3 PUFA into erythrocytes in response to PUFA supplementation. The impact of PLA2 activity on behavioural responses to n-3 PUFA supplementation warrants further evaluation.

Association of Fatty Acids in Buccal Cheek Cells, Red Blood Cells (RBCs) and ω3 Dietary Intake in Children and Adults

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BACKGROUND: Although blood lipids have been employed as the primary indices of fatty acid status, feasibility of obtaining blood samples can be hampered by factors including participant willingness, regulatory restrictions, and staffing. The use of buccal cheek cells as non-invasive, surrogate indices has been explored in infants but use in children and adults is less well documented.

PURPOSE: To evaluate the association of fatty acid profiles in cheek cells to that of RBCs and dietary ω3 fat intake in children and adults.

METHODS: Participants included normal male and female volunteers (n = 68; ages: 7-57yrs) and young males participating in a masked, placebo-controlled, DHA supplementation trial (n=57; ages 9-38yrs). Cheek cells were collected with Dacron swabs, phospholipids isolated and 29 fatty acids quantified as % by GC/FID. Fasting blood samples were collected concurrently and total RBC lipids similarly quantified. A sub-set of normal participants (n=58) completed a 1-page dietary questionnaire (Benisek, AOCS, S85, 2002) estimating ad lib daily intake of EPA and DHA (mg/d).

RESULTS: Significant correlations were found between cheek cell phospholipid (CCPL) and RBC lipid content of EPA (r=0.61, p<0.001), DHA (r= 0.71, p<.001), arachidonic acid (ARA; r=0.23, p=0.01) and 5 of 11 other ω3 and ω6 fatty acids. The mean daily estimated intake of EPA and DHA was 44 mg/d and 78 mg/d, respectively. The intake of EPA and DHA were significantly correlated with CCPL EPA (r=0.67, p<0.001) and DHA (r=0.36, p<0.006) as well as, RBC EPA (r=0.69, p<0.001) and DHA (r=0.64, p<0.001).

CONCLUSIONS: Fatty acid profiles of CCPL provide a surrogate index for most ω3 and ω6 fatty acids in children and adults on diets of varied fat content. Both cheek cells and the single-page ω3 dietary questionnaire are reasonable proxies for monitoring an individual's EPA and DHA status, particularly when blood samples may be unattainable.

Essential roles of lysophospholipid signalings in neuoepithelial cell migration in the developing brain

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The lysophosphatidic acid (LPA), one of membrane lipids, functions as a growth factor that elicits various cellular responses in numerous cell types, including neurons. LPA signals through at least six specific membrane-bound G protein-coupled receptors (GPCRs), LPA1-6. LPA4 is a recently identified LPA receptor known to be involved in the cellular migration of neuronal cells. To date, systematic analysis of LPA4 gene expression and physiological role of LPA4 during brain development has not yet been performed. Previously, our laboratory has characterized LPA4 function in the ventricular zone of the cerebral cortex of developing mice. Here, we report the expression pattern of LPA4 during embryonic brain development using in situ hybridization. In utero electroporation-induced over-expression of the LPA4 gene in the ventricular zone of the cerebral cortex resulted in the malformation of the transfected area of cerebral cortex. This phenotype was similar to human periventricular nodular heterotopia. The

over-expression of LPA4 predominantly destroyed cortical lamination at P0, and migration of neuroblast to outer layer of the cortex were arrested in the intermediate zone in embryonic brain. Extensive confocal microscopic examination revealed that the cortical malformation and neuronal migration defects were due to depletion of neural progenitor pool and to an abnormal neuronal differentiation in developing mouse cerebral cortex. Our results implicate LPA4 as an important receptor in LPA-mediated embryonic brain formation during embryonic development and would provide insights for understanding the pathogenesis of human periventricular nodular heterotopia, and hopes for coping with this hard-to-cure brain disease.

Partial replacement of dietary linoleic acid with alpha linolenic acid attenuate colonic inflammation in a rat model of inflammatory bowel disease

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Ulcerative colitis and Crohn's disease, the two types of inflammatory bowel disease are characterized by recurrent episodes of inflammation and tissue degeneration. Increasing prevalence of inflammatory bowel disease may be due to imbalance in the intake of n-6 and n-3 PUFA in the diet. This study investigates the impact of varying ratios of dietary linoleic acid (LA, 18:2 n-6) to alpha linolenic acid (ALA, 18:3 n-3) on inflammatory response in dextran sulfate sodium induced colitis. Weanling male sprague dawley rats were divided into five groups : a noncolitic group with LA:ALA ratio of 215 and colitic group with LA:ALA ratio of 215, 50, 10 and 2. Blends of groundnut, palmolein and linseed oils were used to provide varying LA:ALA ratios. Total PUFA was kept constant and LA:ALA ratio was altered by substitution of LA with ALA. All the rats were fed the respective experimental isoenergetic diets containing 10% fat for 90 days and dextran sulfate sodium was administered during last 11 days to induce colitis. Colonic inflammation was evaluated by clinical, biochemical and histological parameters. Feeding LA:ALA ratio of 2 reduced the severity of colitis as evidenced by significant reduction in disease activity index, mucosal myeloperoxidase activity ($p<0.05$), alkaline phosphatase activity ($p<0.01$) and increase in colon length ($p<0.01$) compared to the groups fed with higher ratios. This was associated with significant reduction in mucosal proinflammatory cytokines TNF-alpha ($p<0.01$), IL-1beta ($p<0.01$) and improve histological score. Further, ALA supplementation dose dependently increase long chain n-3 PUFA and decreased long chain n-6 PUFA in colon structural lipids. These data suggests that substitution of one-third of LA with ALA (LA:ALA ratio 2) mitigates the experimental colitis by down regulating proinflammatory mediators.

Differential Effects of Dietary Fish Oil and Soy Protein on Renal Disease and Eicosanoids in Chronic Kidney Disease

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Renal prostanoids are elevated in renal disease and pharmacological inhibition of prostanoids can slow disease progression. Since dietary fish oil (FO) and soy protein (SP) also slow disease progression in several models of chronic renal disease (CKD) and can alter renal eicosanoid production, the effects of these dietary interventions on renal disease and eicosanoid formation were determined.

Weanling normal (+/+) and diseased (cy/+) Han:SPRD-cy littermates were given AIN-93G based diets containing either casein protein (CP) or SP, and soy oil (SO) or FO in a 3-way design for 8 weeks. Diseased rats (cy/+) develop renal cysts resulting in progressive CKD.

SP reduced renal cyst growth and fibrosis in both cortex and medulla, while FO reduced fibrosis only in the medulla of diseased rats. Both SP and FO improved serum cystatin c and creatinine in diseased rats with the effect of SP being stronger. Renal eicosanoids, measured by LC/MS/MS, were altered primarily in the diseased compared to normal cortex, with renal prostanoids being increased and hydroxy fatty acids (OHFAs) being generally decreased. SP reduced prostaglandin E2 (PGE2) and metabolites of prostacyclin and thromboxane A2 in the diseased cortex in parallel with its protective effects on disease. 5-hydroxyeicosatetraenoic acid (5-HETE), and 13-hydroxyoctadecadienoic acid (13-HODE) were reduced in diseased kidneys and SP increased their levels in the cortex. On the other hand, FO reduced all prostanoids, most OHFAs, and increased formation of 3-series prostanoids, with effects being observed in both normal and diseased cortex and medulla.

Hence, FO effects on renal eicosanoids did not parallel effects on disease, which were observed only in the medulla. On the other hand, SP opposed the effects of disease on renal cortical prostanoids, 5-HETE and 13-HODE levels in this CKD model. SP may mediate its renoprotective effects via blunting of these disease induced alterations.

Postprandial metabolism of n-3 fatty acids following a single meal with different vegetable oils

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Saturated, monounsaturated and n-6 and n-3 fatty acids vary in esterification in triglycerides, phospholipids and cholesterol esters, as well as oxidation for energy and further metabolism. Alpha linolenic acid has been suggested to be rapidly oxidized, raising questions as to whether utilization by the metabolically active intestine may contribute to low circulating 18:3n-3, hence contributing to low efficacy as a substrate for desaturation. We evaluated the postprandial appearance and clearance of fatty acids from unsaturated vegetable oils when consumed in meals balanced for fat, carbohydrate and protein. Healthy men consumed meals with high oleic safflower, soybean, canola or flax oil with blood collected at 0, 1, 2, 4 and 6 hours. Chyomicrons were collected then lipoproteins prepared using self-generating iodixanol gradients. Lipid classes were separated by HPLC and fatty acids determined by GLC. Men consuming flax oil showed an early rise in chylomicron triglyceride (TG) 18:3n-3 to 28+1.5% by 2 hours, maintained to 6 hours postprandial, with a drop in 16:0 from 22.6+1.3l to 15.6+1.2%, while18:2n-6 remained high at 18-20%. Canola and soybean oil had similar 9.3 and 8.9% 18:3n-3, respectively, with 18:2n-6/18:3n-3 ratios of 2.06 and 6.03 in the two oils, respectively. Chylomicron 18:3n-3 increased to 6.0+0.25 and 4.83+0.24% at 4 hours post-prandial, with13.4+1.05 and 19.1+0.91% 16:0, and 17.7+0.23 and 33.8+1.35% 18:2n-6 at 4 hours after consuming canola or soybean oil, respectively. The chylomicron 18:2n-6/18:3n-3 showed dramatic changes, with a decline from 8.4 to 3.0 in men consuming canola oil, to 7.1 in men consuming soybean oil, and to 0.8 in men consuming flax oil. Overall, 18:3n-3 absorption is rapid, reaching high levels by 2 hours postprandial, preceding peak absorption of 18:2n-6, and with marked effects on the balance of 18:2/18:3n-3 in postprandial chylomicrons TG delivered as metabolic substrates to organs. Supported by Canola and Flax Councils of Canada.

Effect of chronic administration of arachidonic acid on skeletal muscle lipids in aged rats

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Arachidonic acid (20:4n-6, AA) is a polyunsaturated fatty acid that synthesized from linoleic acid (18:2n-6) in the many tissues. AA is a major constituent of the cell membrane, thus playing an important role in the maintenance of physiological functions. Skeletal muscle mass declines with aging, as does the potential for overload-induced fast-twitch skeletal muscle hypertrophy. AA is necessary for the repair and growth of skeletal muscle tissue. AA is a regulator of localized muscle inflammation, and may be a central nutrient controlling the intensity of the anabolic/tissue-rebuilding response to weight training. In the present study, we examined the effects of chronic administration AA on fatty acid composition and lipid peroxidation of skeletal muscles in aged rats. Aged male rats (21 months old) were divided into two groups, which was orally administered AA-enriched triacylglycerol (AA: 240 mg/kg/day) for 13 weeks. Fatty acid composition in slow and fast twitch muscles was measured by gas chromatography and the level of lipid peroxide (LPO) by the thiobarbituric acid-reactive substances assay. In plasma, total cholesterol and creatinine levels tended to increase, and low and high density lipoprotein cholesterol were significantly increased with AA administration. In slow twitch muscles, the ratio of n-6 to n-3 was increased, and inversely DHA, nervonic acid and the ratio of DHA to AA were decreased with AA administration. On the other hand, the levels of AA and LPO were not affected. In fast twitch muscles, AA was increased, and inversely linoleic acid and the ratio of DHA to AA were decrease with AA administration. The level of LPO tended to increase. These results suggest that effect of chronic administration of AA differ in types of muscular fiber in aged rats.

Dietary fat manipulation has a greater impact on postprandial lipid metabolism than the apolipoprotein E (epsilon) genotype – insights from the SATgenε study

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Non-fasting plasma triacylglycerol (TAG) is being increasingly recognised as a cardiovascular disease risk factor. The aetiology of the highly heterogenous response of plasma lipids to dietary fat manipulation is relatively unknown. Our aim was to determine the effects of chronic dietary fat manipulation on postprandial lipaemia according to apolipoprotein (APO)E (epsilon) genotype. Participants (mean age 53 (SD 9) y and BMI 25.8 (SD 2.9) kg/m2) prospectively recruited according to APOE genotype (n=12 E3/E3, n=11 E3/E4), followed a sequential dietary intervention in which they were assigned to a low fat (LF), high fat-high saturated fat (HSF), and HSF diet with 3 g/d docosahexaenoic (HSF-DHA) each for an 8 wk period, in the same order. At the end of each dietary period, a 480 min postprandial assessment was performed using a test meal with a macronutrient profile representative of the previous dietary intervention. Blood samples were collected for the measurement of plasma metabolites, and for the isolation of TAG-rich lipoprotein fractions (Svedberg flotation rate (Sf)>400, Sf 60-400 and Sf 20-60). Relative to APOE genotype, dietary fat manipulation had a greater impact on lipids, with a lower fasting TAG in plasma and Sf 60-400 TRL fraction, cholesterol in the Sf 20-60 fraction, and plasma NEFA following the HSF-DHA than LF and HSF interventions (P≤0.007). These differences in fasting concentrations were reflected in the magnitude of the postprandial responses after the meals. However, a variable postprandial plasma TAG response according to APOE genotype was evident, with a lower area under the curve and peak concentration after HSF-DHA compared to the LF (23%) or HSF (29%) diet/test meals in APOE4 carriers (P≤0.005). In conclusion, although a modest impact of APOE genotype was observed on the plasma TAG profile, dietary fat composition emerges as a more important modulator of the postprandial response.

Investigation of the effects of DHA-rich fish oil and Efalex Active 50+ on cerebral haemodynamics in healthy adults aged 50-70 years reporting subjective memory deficits: Preliminary results

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Background: Age-related cerebral insufficiency has been shown to be ameliorated following administration of DHA in monkeys (Tsukada et al. 2000), and data from our own lab has demonstrated task-related increases in cerebral blood flow following 12 weeks' administration of DHA-rich fish oil (FO) in healthy young adults (Jackson et al. 2011). The effect of administration of DHA-rich FO, or FO in combination with other compounds in older adults has yet to be explored.

Objective: To evaluate the cerebral haemodynamic effects of DHA-rich FO and DHA-rich FO with added phosphotidylserine, Ginkgo biloba, folic acid and vitamin B12 (Efalex Active 50+) using Near Infrared Spectroscopy in healthy older adults aged 50-70 years reporting subjective memory deficits. As a secondary measure performance on cognitive tasks was assessed.

Procedure: Relative changes in the concentration of oxyhaemoglobin and deoxyhaemoglobin were assessed using Near Infrared Spectroscopy (NIRS) during the performance of cognitive tasks prior to and following 26 weeks' daily administration with either DHA-rich FO (896 mg DHA + 128 mg EPA), Efalex Active 50+ (946.4 mg DHA + 160 mg EPA, 88 mg phosphatidylserine, 240 mg Ginkgo biloba, 1 mg folic acid, 24 mg vitamin B12) or placebo (high oleic acid sunflower oil).

Results: Preliminary results from a subset of data (n=59) show a pattern of increased cerebral blood flow as measured by NIRS following both active treatments compared to placebo, however this result did not reach significance. There was a main effect of treatment on performance of an attention task, with post hoc tests revealing poorer performance following FO compared to both placebo and Efalex Active 50+; however participants who received FO were faster than the both treatment groups on the same task.

Conclusion: Evidence of increased cerebral blood flow following administration of either FO or FO in combination with other compounds in older adults was not found in this sample subset.

Dietary Fish Oil Decreases Inflammatory Infiltrate and Epithelial Proliferation in Mice with C rodentium Induced Colitis

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Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis are idiopathic, chronic intestinal inflammatory disease characterized by frequent relapses and remission. Dietary n-6 and n-3 fatty acids are major, modifiable pleiotropic environmental factors that may contribute to exacerbation or quiescence of IBD symptoms. Distinct from models of inflammation involving chemically-induced colitis, Citrobacter rodentium infection offers an ideal approach to investigate the role of dietary fatty acids in pathogen-host interactions in the intestine. Mice were fed, as a percent energy, 15% 18:2n-6 and <0.1% 18:3n-3 (safflower oil), or 4% 18:2n-6 + 2% 18:3n-3 (canola oil), or 5% DHA + 1.5% EPA with 2% safflower oil, with constant total fat and other nutrient intakes. Mice were then infected orally with C. rodentium and studied at 10 days post-infection. Mice fed safflower oil had lower n-3 and higher n-6 fatty acids in colonic phospholipids than mice fed canola or fish oil. Colonic histological damage decreased and the number of macrophages and neutrophils increased in groups in order safflower oil> canola oil> fish oil. Immuno-flourescence staining showed mice fed fish oil prior to and during infection had significantly higher colonic epithelial cell Ki67 positive cells, a nuclear factor that marks cell proliferation. PCR analyses of gene expression showed lower IL-6 and IL-10 gene expression in fish oil fed mice. Overall, these studies show dietary fish oil lessened and safflower oil exacerbated the severity of C. rodentium induced colitis through alteration of the host mucosal immune responses.

The potential of omega-3 polyunsaturated fatty acids on delaying cognitive decline in elderly people

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Cognitive function is a major determinant of quality of life in old age and decline in cognitive functioning is a major socio-economic and healthcare concern. There is a recent increasing interest and focus on the potential of omega-3 polyunsaturated fatty acids (n-3 PUFA) on measures of cognitive outcomes in aging people with normal cognition , age-related cognitive decline(ARCD), and cognitive impairment of both degenerative(mild cognitive impairment, MCI; Alzheimer's disease, AD) or vascular origin. We systematically reviewed the published literatures searched for in computerized databases with intent to conduct a meta-analysis. At present, several randomized controlled trials (RCT) suggested that an increase of n-3 PUFAs intake (DHA, EPA or fish oils) could not only increase the cognitive ability of aging people with MCI, but also slow cognitive decline in cognitively healthy older adults and ARCDs. However, beneficial effects of elevated n-3 PUFAs intake on cognitive function in AD were not found in RCTs. Cross-sectional and prospective studies concerned with the association between diets and cognitive decline suggested a positive association between n-3 PUFAs intake and cognitive outcomes, delaying the onset of dementia, both of degenerative or vascular origin. The available data from studies are insufficient to draw strong conclusions about the protective effects of n-3 PUFAs against risk of AD and dementia. However, limited evidence suggested that appropriate dietary measures or supplementation with n-3 PUFAs might open new ways for the prevention and management of mild cognitive decline which is possibly the earliest stage of detectable AD. Therefore, larger sample sizes work is needed to identify the association and the possible neurobiological mechanisms underlying it.

Keywords: cognition; cognitive function; aging; MCI; AD; ARCD; omega-3 fatty acid; RCT

Stability Blended Oil Enriched with DHA&EPA in Different Cooking Methods

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Inadequate intake of n-3 polyunsaturated fatty acids (PUFA) has received increasing attention for their health aspects. More and more authority organizations have recommended daily intake of PUFA, especially eicosapentanoic (EPA) and docosahexanoic (DHA) acids. However Chinese average daily

intake of DHA&EPA is 37.6 mg/person according to Chinese Nutrition and Health Survey conducted in 2002. More and more food enriched with PUFA has been developed recently to overcome this insufficiency. However, susceptibility of oxidation of PUFA is the first issue for developing such food.

In this case, a blended oil enriched with DHA&EPA was designed and its stability during storage and cooking was investigated. This blended oil contained 4000 ppm DHA&EPA.

Deep frying and stir-frying were adopted in this study to investigate the cooking stability of DHA&EPA. A very traditional cooking method-stirring frying was studied with this blended oil. After stir-frying, acid value, peroxidant value and p-anisidine value were measured. After cooking, the oil remained quite fresh and no significant difference was found for the blended oil with fish oil and the control. At least 95% DHA&EPA could be kept in oil. Frying of French fryers was also conducted for 24 hours in 6 days. All the oxidative indices measured like those in stir-frying had no significant difference between the oil with fish oil and the control. After 5 days frying, the frying oil quality can still meet the frying oil hygienic standards of Australia, Netherland, Belgium, US, Japan and China. However, about 8% DHA&EPA were lost after each days frying. The total loss of DHA&EPA after 24 hours is 48%. In this case, the blended oil with fish oil is very stable in stir-frying and it is not recommended for restaurant or food industry frying.

Key words: Docosahexaenoic acid (DHA) , eicosapentaenoic acid, blended oil; stability

Distinct Fatty Acid Profile in Diet and Breast Milk of Farming Women

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Farmers and their children have lower incidence of atopic sensitization and allergy. Contact with livestock appears to be a strong protective factor, but differences in diet may also play a role. Decreased consumption of butter (rich in saturated fatty acids) and increased consumption of margarine (rich in polyunsaturated fatty acids) have been associated with atopic disease. The aim of this study was to identify dietary patterns among farming families that could be related to protection against allergy, and also to investigate if dietary intake of fatty acids was reflected in breast milk. The study enrolled a birth-cohort of 28 children who grew up on farms and 37 children who lived in the same rural region but not on farms. Dietary data for mothers were collected during pregnancy and lactation at 1 and 4 months postpartum. Umbilical cord blood and serum at 4 months postpartum were obtained for fatty acid analysis. Maternal breast milk obtained at 1 and 4 months postpartum and maternal serum 1 month postpartum were also analyzed for fatty acids. Farming women reported higher intake of butter, whole milk and whole-fat cream compared with non-farming controls. All of these dairy products have a high content of saturated fatty acids, and accordingly, breast milk from farming women had higher levels of 18:0. Conversely, non-farming control women consumed more margarine rich in polyunsaturated fatty acids and low-fat dairy products, which was reflected in their breast milk as higher levels of the polyunsaturated fatty acids linoleic and α-linolenic acid compared with farming women. In conclusion, our results may suggest that a paucity of unsaturated fatty acids in the diet on farms, including the breast milk consumed by infants of farming mothers, may be one factor explaining the allergy-preventing effect of a childhood on dairy farms.

Supplementation of arachidonic acid-enriched oil increases arachidonic acid contents in plasma phospholipids, but does not increase their metabolites and clinical parameters in Japanese healthy elderly individuals: a randomized controlled study

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Background: The importance of arachidonic acid (ARA) among the elderly has recently gained increased attention. The effects of ARA supplementation in the elderly are not fully understood, although ARA is considered to be associated with various diseases.

Objective: We investigate whether ARA supplementation to Japanese elderly subjects affects clinical parameters involved in cardiovascular, inflammatory, and allergic diseases. We also examine levels of ARA metabolites such as prostanoids during intervention.

Methods: We conducted a randomized, double-blind and placebo-controlled parallel group intervention trial. ARA-enriched oil (240 or 720 mg ARA per day) or placebo was administered to Japanese healthy men and women aged 55-70 years for 4 weeks followed by a 4-week washout period. The fatty acids contents of plasma phospholipids, clinical parameters, and ARA metabolites were determined at baseline, 2, 4, and 8 weeks.

Results: The ARA content in plasma phospholipids in the ARA-administrated groups increased dose-dependently and was almost the same at 2 weeks and at 4 weeks. The elevated ARA content decreased to nearly baseline during a 4-week washout period. During the supplementation and washout periods, no changes were observed in eicosapentaenoic acid and docosahexaenoic acid contents. There were no changes in clinical blood parameters related to cardiovascular, inflammatory and allergic diseases. ARA supplementation did not alter the level of ARA metabolites such as urinary 11-dehydro thromboxane B2, 2,3-dinor-6-keto prostaglandin (PG) F1α and 9,15-dioxo-11α-hydroxy-13,14-dihydro-2,3,4,5-tetranor-prostan-1,20-dioic acid (tetranor-PGEM), and plasma PGE2 and lipoxin A4. ARA in plasma phospholipids was not correlated with ARA metabolite levels in the blood or urine.

Conclusion: These results indicate that ARA supplementation, even at a relatively high dose, does not increase ARA metabolites, and suggest that it does not induce cardiovascular, inflammatory or allergic diseases in Japanese elderly individuals.

Effect of Krill oil on learning and memory and brain biochemistry

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Krill oil is obtained from Antarctic krill (Euphausia superba Dana). Krill oil contains about 45% phsopholipids, of which 95% is phosphotidyl choline. The phospholipids in Krill oil are rich in EPA and DHA. Phospholipid bound LC-PUFAs are better transported to brain. We hypothesized that Krill oil will help improve the memory. To test the hypothesis, we selected the male Wistar rats and employed radial 8 arm maize as the model for testing for memory.

The rats were kept on fish oil free diet. Inbred second generation rats, 25 weeks old, were divided into 3 groups: high-dose KO (HD; 300 mg EPA, 120 mg DHA), low-dose KO (LD; 215 mg EPA, 86 mg DHA), and the control group that received sterilized water only. The supplementation in diets were started 6 weeks before the training and testing (3 weeks) for radial maize arm began and continued through out the protocol. A subgup of rats from control and high dose krill oil were injected with 5-bromo-2′-deoxyuridine (BrdU) for 5 days to study for generation of new neuronal cells.

Krill oil treatment resulted in increases in plasma EPA, DPA and DHA and a reduction in AA. In hipoocampus and cortex, DHA increased while AA levels decreased, leading to increase in ratio of DHA/AA. Lipid peroxide levesl were decreased in brain tissues (cortex and hippocampus). BrDU staining indicated increase n new neuronal cell formation in hippocampus and cortex. Both parameters of radial maize test, reference memory error (RME) and working memeory error (WME) decreased.

The results of this study indicate that krill oil treatment increased palsma and brain tissue levels of DHA, reduced levels of AA and lipid peroxidation. These changes were associated with reduced memory errors, indicating postive effect of krill oil on learning and memory in the selected model.

Omega-3 Enrichment and Sensory Properties of Eggs of Two Strains of Laying Hens Fed High alpha-Linolenic Acid Diets

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Including fish oil in the diet increases the omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) in eggs, but it can result in fishy flavours. The aim of the study was to evaluate the effects of including a vegetable source of n-3 fat in the form of alpha-linolenic acid (ALA, 18:3n-3) in the diets of two strains of laying hens on production performance, n-3 LCPUFA accumulation and sensory properties of eggs. Forty eight hens (24 Hy-Line white and 24 Hy-Line brown) were randomly assigned into 3 dietary treatments. The ALA levels of diet varied from 0.3 to 6% energy (%en) while the level of the n-6 fatty acid, linoleic acid (LA, 18:2n-6) was held constant to less than 5%en in all diets. Birds were placed at point of lay and fed ad libitum for 12 weeks. Results showed that the incorporation of vegetable oils rich in n-3 PUFA (ALA) did not modify feed intake, feed conversion ratio, egg production and egg weight of laying hens. Incorporation of vegetable oils rich in n-3 PUFA (ALA) increased EPA, DPA, DHA and total n-3 levels of the eggs. Enriching ALA levels in the diets had no effect on aroma, taste, egg flavour or off flavours of boiled eggs. Increased ALA levels of the diet did not change the consumer acceptance of the eggs compared with eggs purchased from a local supermarket. In conclusion, feeding diets rich in ALA to laying hens had no effect on production performance of birds or sensory properties of eggs. As birds fed high ALA diets produced eggs higher in n-3 LCPUFA, this provides an alternative n-3 rich food for consumers.

Conjugated linolenic acid controls neuronal differentiation of cultured neural stem cells by alternating mRNA levels of bHLH transcription factors and cell cycle

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Background   Polyunsaturated fatty acids (PUFAs) could enhance neurogenesis and it might be helpful to recover from neuronal diseases such as Alzheimer's disease and depression. However, the exact mechanisms of the beneficial effects of PUFAs on neurogenesis have not been conclusively described. We recently demonstrated that docosahexaenoic acid (DHA) induces neuronal differentiation by decreasing Hes1 expression and increasing p27 expression, which causes cell cycle arrest in cultured neuronal stem cells (NSCs). In this study, we examined the effect of arachidonic acid (AA), linolenic acid (LA) and conjugated linolenic acid (CLA) on neuronal or glial differentiation, expression of basic helix-loop-helix transcription factors (Hes1, 6 and NeuroD) and the cell cycle of cultured NSCs in a differentiation medium.

Methods   NSCs were collected from E14.5 rat fetal forebrain and cultures as neurosphere in the bFGF2 containing media. Neurospheres were collected and dissociated before PUFA treatment. NSCs were seeded and treated with PUFAs (AA, LA and CLA) in 0.01% BSA containing media. PUFA treated cells were collected and examined mRNA expression level by real-time PCR, immunofluorescent staining (Tuj-1) and cell-cycle by Flow Cytometry.

Results   CLA increased the number of Tuj-1 (a neuronal marker) positive cells at 4 or 7 days after treatment, indicating that CLA induced neuronal differentiation. CLA also increased Hes6, an inhibitor of

Hes1, and MAP2 mRNA. AA and LA did not affect MAP2 mRNA level and number of Tuj-1 positive cells. CLA increased the mRNA levels of p21 and p27, a cyclin-dependent kinase inhibitor and decreased number of S-phase cells. These results indicate that CLA could be involved in neuronal differentiation by different mechanisms from DHA. LA and AA did not affect neuronal differentiation in cultured NSCs.

Dietary modification of polyunsaturated fatty acids: effect on plasma and erythrocyte membrane lipid composition

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Omega-3 fatty acids play an important role in membrane function and cytokine modulation. Approximately 20% of the dry weight of the brain is made up of polyunsaturated fatty acids of which arachidonic acid and docosahexaenoic acid are the major constituents. The composition of phospholipids in cell membranes alters many membrane functions such as protein and ion transport. There is also an increasing body of evidence that supports the role of long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) in cognitive function.

The fatty acid composition of cell membrane phospholipids reflect dietary intake. Recent Irish data shows that dietary intakes of LC n-3 PUFA are below international recommendations.

The aim of this study was to determine the effect of 6 month dietary supplementation with eicosapentaenoic acid (EPA) (1120mg daily) on the erythrocyte membrane fatty acid composition. Following a double blind randomised controlled trial (RCT) design, healthy individuals (18-65y) were randomised to receive a dietary supplement (1120mg EPA & 36mg gamma- linolenic acid) or placebo (refined sunflower oil) for 24 weeks. Basal dietary intake of LC n-3 PUFA was assessed by 12-day food diary and food frequency questionnaire DIETQ, analysed by WISP (Tinuviel Software, UK).

The fatty acid profile of plasma and the phosphatidylcholine, triacylglycerol, non-esterified fatty acids and cholesterol esters fractions of the erythrocyte membrane were determined by LC/MS at baseline,12 and 24 weeks of supplementation. The data from this study will provide the basis for discussion of the time-course and magnitude of change in the fatty acid composition of a representative membrane in response to dietary intervention.

Olive oil (refined)-enriched diet upregulates avian uncoupling protein expression and proton leak in the skeletal muscle mitochondria

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We have previously found that olive oil-supplemented (+6.7%) diet increases avian uncoupling protein (avUCP) content and reduces mitochondrial reactive oxygen species (ROS) production in bird's skeletal muscle. In the study, however, it could not be ruled out that these changes result from high caloric intake rather than the olive oil intake. This study clarifies i) whether dietary olive oil can be one of important determinants of avUCP expression and ROS production in muscle mitochondria and ii) how the ROS production can be reduced in the olive oil-fed birds. 10-d-old chickens were fed diets containing either soybean oil (control) or refined olive oil with two different levels for 10 days. In birds fed diets containing 6% fat, there was no difference in avUCP gene expression in skeletal muscle between control and olive oil-fed groups, while, in birds fed diets containing 12.7% fat, the avUCP expression was higher in olive oil-fed group than in control group. In addition, there was little difference in the avUCP expression between the birds fed diets containing 6% and 12.7% soybean oil. These results indicate that increase in muscular avUCP expression is not due to high caloric intake, but to high levels of olive oil intake. In agreement with the increased avUCP expression, avUCP-stimulated proton leak was higher in 12.7% olive oil-fed group than in 12.7% control group. In birds fed the diets containing 12.7% fat, mitochondrial ROS production during basal proton leak had a tendency to decrease in olive oil-fed group than in control group, though there was little difference in the ROS production during avUCP-mediated proton leak between those

groups. From these results, it can be concluded that alterations in the ROS production due to dietary olive oil might be involved in not only avUCP expression but also membrane composition in skeletal muscle mitochondria.

NEFA determination in human plasma: comprehensive UPLC-MS/MS quantitation

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Objectives: Nonesterified fatty acids (NEFA) are highly associated with various diseases like obesity, insulin resistance, diabetes, adrenoleukodystrophy or schizophrenia. Consequently, NEFA are potential biomarkers for alterations in lipid metabolism and pathological outcomes. Nevertheless, straightforward quantitative methods for determination of NEFA species are still missing.

Material and methods: For reducing analysis time, a sample preparation was utilized avoiding cumbersome and solvent-consuming extraction or derivatization procedures. Short run time with high resolution was achieved by UPLC separation coupled to LC-MS/MS detection. The implementation of qualifier ions supported the unequivocal determination of NEFA.

Results: 36 NEFA species were quantified in healthy human plasma, the highest numbers ever reported for a LC-MS application. Sample preparation is fast and non-expensive. In combination with automated liquid handling, total assay time per sample is less than 15 minutes for 96 well plates. In addition, benefit was achieved by implementing a prediction model to determine numerous NEFA by predicting all relevant analytical parameters of NEFA species based on chain length and number of double bonds. Recovery and precision were in the acceptable limits of 80-120% and < 15%, respectively.

Conclusion: The protocol presented here provides unbiased and comprehensive quantitation of plasma NEFA species by LC-MS/MS in human plasma. This enables application in clinical trials with high sample number which have to be analyzed in short time and with low costs.

Reversed Phase LC/MS/MS Method for Targeted Quantification of Glycerophospholipid Molecular Species in Plasma

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Objective: The relationship between lipid status and metabolism, infant development and health has widely been studied, but the importance of individual glycerophospholipid species for biological functions in infants has hardly been considered. We developed a method for quantitative analyses of plasma glycerophospholipids from small sample volume.

Material and methods: Proteins were precipitated with methanol, which eliminated further sample preparation. The supernatant was analyzed by reversed-phase HPLC using a gradient of water, methanol and isopropanol as mobile phase. Electrospray ionisation in negative mode in combination with tandem mass spectrometry enabled detection of specific fatty acids as fragments of glycerophospholipid species.

Results: With this combination of chromatography and mass spectrometry, PC, lyso-PC, PE and lyso-PE species and there relevant isobaric compounds were quantified. Method validation showed a linear working range between 0.05 μmol/L and 10 μmol/L in diluted plasma samples. The intra-assay coefficients of variation (n = 6) ranged from 1.1% to 13.9%. Results were comparable with data of the human metabolome database and gas chromatographic fatty acid analyses.

Conclusion: All quantitatively important PE and PC species are covered. The method can be applied for investigating dietary effects on plasma GP composition from small plasma volumes.

Differential Effects of Rosiglitazone and Pioglitazone in the Combination Treatment with n-3 Fatty Acids in Mice Fed a High-Fat Diet

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Background/Objective: Combining pharmacological treatments and life style interventions is necessary for effective therapy of diseases clustered in metabolic syndrome. Acting via multiple mechanisms, combination treatments may reduce dose requirements and, therefore, lower the risk of adverse side effects associated with long-term pharmacological interventions. Our previous study (Kuda et al, Diabetologia 2009) in mice fed high-fat diet indicated additivity/synergism in preservation of insulin sensitivity and in amelioration of major metabolic syndrome phenotypes by the combination treatment using long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) and a low dose of a thiazolidinedione (TZD) anti-diabetic drug, namely rosiglitazone. We investigated here whether pioglitazone, a TZD-drug in clinical use, could also elicit the additive beneficial effects when combined with LC n-3 PUFA.

Procedures: Adult male mice (C57BL/6N) were fed an obesogenic corn oil-based high-fat diet (cHF) for 8 weeks, or randomly assigned to various dietary treatments (i) cHF+F, cHF with LC n-3 PUFA concentrate replacing 15% of dietary lipids; (ii) cHF+ROSI, cHF with 10 mg rosiglitazone/kg diet; (iii) cHF+F+ROSI; (iv) cHF+PIO, cHF with 50 mg pioglitazone/kg diet; (v) cHF+F+PIO, or chow-fed. Plasma concentrations of 163 metabolites were evaluated using a targeted metabolomics approach.

Results: Both TZDs preserved glucose homeostasis and normal plasma lipid levels while inducing adiponectin, with pioglitazone showing better effectiveness. The beneficial effects of TZDs were further augmented by the combination treatments. cHF+F+ROSI but not cHF+F+PIO counteracted development of obesity, in correlation with inducibility of fatty acid beta-oxidation, as revealed by the metabolomic analysis. By contrast, only cHF+F+PIO eliminated hepatic steatosis and this treatment also reversed insulin resistance in dietary obese mice.

Conclusion: Our results reveal surprisingly different effects of rosiglitazone and pioglitazone, unmasked in the combination treatment with LC n-3 PUFA. They support the notion that n-3 LC-PUFA could be used as add-on treatment to TZDs in order to improve diabetic patient's therapy and even to reduce obesity.

An Intron 1 Insertion/Deletion (Indel) Polymorphism in the Fatty Acid Desaturase 2 (FADS2) Gene is Associated with HUFA Synthesis in Cancer Cell Lines

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Background: Genes coding for the fatty acid desaturases (FADS1 and FADS2) localized to human chromosome 11q13 cancer hotspot locus are required for highly unsaturated fatty acid (HUFA) biosynthesis. In several cancer cell lines the rate limiting step catalyzed by FADS2 gene product is not functional. Recently, we showed that FADS2, upon heterologous expression in MCF-7 breast cancer cells, restores Δ6 and Δ8-desaturase activity and normal arachidonic acid and eicosapentaenoic acid synthesis. Genetic variants in protein coding or non-coding sequences associated with FADS2 loss have not been described. We sequenced the coding regions along with a conserved portion of intron 1 flanking a sterol regulatory element (SRE) that we show (elsewhere) mediates enhanced HUFA biosynthesis in response to simvastatin, as well as a deletion variant region 5' upstream, to characterize the molecular defect associated with loss of FADS2 enzymatic function. Methods: DNA and RNA were extracted from seven human cancer cell lines (MCF-7, Caco-2, HepG2, SK-N-SH, Jurkat, HeLa, Y79). The coding region flanking the SRE, and the 5' upstream variant were amplified using sequence-specific primers. The products were gel eluted and sequenced. Results: No differences compared to the reference human genome were found in the protein coding or 5' upstream regions. However, the fragment flanking the SRE DNA sequence showed >20 base pair deletion downstream of the SRE binding site. HepG2, SK-N-SH, Jurkat, and Hela had one copy of the deletion, whereas Caco-2 and MCF-7 were homozygous for the deletion. Y79 retinoblastoma cells, known to biosynthesize docosahexaenoic acid, lacked the deletion.

Conclusions: Recent data provide evidence of the existence of well conserved regulatory elements in intronic regions. As the activities of desaturases appear to be regulated at the transcriptional level, the association of cancer cells deletion and HUFA synthesis suggests that this region might be critical for FADS2 gene functionality.

Identification of an insertion-deletion polymorphism in FADS2 intron 1 associated with SREBP binding that upregulates FADS1 and mediates enhanced HUFA biosynthesis in response to simvastatin and LXR agonists

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The fatty acid desaturase genes (FADS1 and FADS2) code for enzymes required for synthesis of omega-3 and omega-6 highly unsaturated fatty acids (HUFA) important in the central nervous system, inflammatory response, and cardiovascular health. Single nucleotide polymorphisms (SNPs) in these genes are associated with numerous health outcomes, but it is unclear how genetic variation affects enzyme function. Here, lymphoblasts obtained from Japanese participants in the International HapMap Project were evaluated for association of expression microarray results with SNPs in the FADS gene cluster. Six SNPs in the first intron of the FADS2 gene were significantly associated with FADS1 expression after Bonferroni correction. A 10-SNP haplotype in FADS2 (rs2727270 to rs2851682, permuted p = 0.028) present in 24% of the population was significantly associated with lower expression of FADS1. A highly conserved region coinciding with the most significant SNPs contained predicted binding sites for PPARγ and SREBP. Lymphoblasts homozygous for either the major or minor haplotype were treated with agonists for these transcription factors, and expression of FADS1 and FADS2 was measured. The statin drug simvastatin and the LXR agonist GW3965 both upregulated expression of FADS1 and FADS2; no response was found for PPARγ agonist rosiglitazone. Surprisingly, minor haplotype homozygotes had 20- 40% higher induction of FADS1 and FADS2 after simvastatin or GW3965 treatment. All minor haplotype carriers had two deletion mutations within 200 bp of the putative sterol response element binding site, and none were found among the major haplotype carriers. These data suggest that genetic elements associated with the minor haplotype may render carriers particularly vulnerable to alterations in diet that result in low HUFA intake and limited intake of linolenic acid. These individuals may be especially responsive to statin or omega-3 HUFA supplementation.

FADS2 Function Loss at the Cancer Hotspot 11q13 Locus Diverts Lipid Signaling Precursor Synthesis to Unusual Eicosanoid Fatty Acids

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The genes coding for the fatty acid desaturases (FADS1, FADS2, FADS3) are located on the long arm (q) of human chromosome 11, region 1, at the 2 and 3 bands (11q12-13.1). 11q13 emerged two decades ago as a hotspot for breast and colon cancer, results that have been confirmed in numerous studies including recent ones. In several cancer cell lines, FADS2-encoded Δ6 and Δ8 desaturation is not functional. Methodology/Principal Findings. MCF7 human breast cancer cells were analyzed with detailed structural mass spectrometry that reveals the position of double bonds in highly unsaturated fatty acids (HUFA). In normally cultured cells absent Δ6-desaturase activity, unusual butylene-interrupted HUFA are observed, probably by the action of the FADS1-encoded Δ5-desaturase on the linoleic acid and linolenic acid elongation products via 11,14-20:2 → 5,11,14-20:3 and 11,14,17-20:3 → 5,11,14,17-20:4. These PUFA are missing the 8-9 double bond of the eicosanoid signaling precursors arachidonic acid (5,8,11,14-20:4) and eicosapentaenoic acid (5,8,11,14,17-20:5). We tranfected normal FADS2 classical transcript to MCF7 cells and the resulting heterologous expression of FADS2 restored Δ6 and Δ8-desaturase activity and normal HUFA composition. Conclusions/Significance. These results demonstrate that the upstream and downstream activities operate and that the molecular defect resides in the FADS2 gene itself. The unusual butylene interrupted HUFA 5,11,14-20:3 and 5,11,14,17-20:4 are not substrates for cyclooxygenase or other enzymes catalyzing the synthesis of eicosanoid signalling molecules via addition of oxygen at or near the 8-9 double bond. Thus, the loss of FADS2-encoded

activities in cancer cells shuts down normal PUFA biosynthesis, deleting the endogenous supply of eicosanoid and downstream docosanoid precursors, and replacing them with unusual butylene-interrupted fatty acids. If recapitulated in vivo, the normal eicosanoid and docosanoid cell signaling milieu would be depleted and altered due to reduction and substitution of normal substrates with unusual substrates, with unpredictable consequences for cellular communication.

Vegetable ALA and SDA-rich Echium oil effectively increased EPA and DPA in blood fractions and decreased serum triacylglycerols in humans

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Aim: The objective of this study is to investigate the conversion of the land-based n-3-LC-PUFA precursors ALA and SDA into EPA, DPA and DHA in humans of different age and BMI by oral supplementation of Echium oil.

Design: The Echium oil from E. plantagineum (CRODA) was supplemented to the diet (5 g ALA and 2 g SDA) to investigate the accumulation of n-3-LC-PUFA (EPA, DPA, DHA) in blood fractions (plasma, erythrocytes, peripheral blood mononuclear cells; PBMC) during an eight weeks period. Three test groups with different mean age, BMI and baseline blood lipids were included. The results were compared with the supplementation of 2 g EPA/d (positive control; n=20). All subjects consumed no n-3-rich foods, e. g., sea fish and flaxseed oil (10 wks).

Results: All subjects (n=60; group 1, 28 y & BMI 22; group 2, 59 y & BMI 23; group 3, 60 y & BMI 30) showed higher portions of fatty acids from Echium oil (ALA and SDA) in plasma, erythrocytes and PBMC. Furthermore, their endogenous elongation and desaturation products ETA, EPA and DPA were increased (2- to 3-fold), independent of age and BMI. However, the DHA was unchanged in plasma and erythrocytes while the DHA even decreased in PBMC.

The serum total cholesterol, LDL-cholesterol and triacylglycerol concentrations were significantly decreased after the Echium oil supplementation, especially in those subjects with (pre-)metabolic syndrome. Those subjects had an BMI at 30, a higher waist circumference (104 cm) and a higher body fat mass (32 %), which was associated with higher mean blood lipid concentrations at baseline (e.g., triacylglycerols 1.79 mmol/L).

Conclusion: The daily consumption of 17 g Echium oil can improve the n-3-LC-PUFA status in humans and the concentration of cardiovascular risk factors in serum, such as LDL-cholesterol and triacylglycerols, were decreased, especially in subjects with increased risk.

Fatty acid composition of adipose triglycerides as markers of weight loss and maintenance: Diogenes project

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Background: Fatty acid (FA) composition of adipose triglycerides (ATG) reflects composition of dietary fat but also metabolic processing eg. endogenous lipogenesis and oxidation of fatty acids. FA composition of ATG in obesity is influenced by weight changes. Palmitoleic acid was shown as possible marker of endogenous lipogenesis.

Objective: To assess the relationship of adipose fatty acid composition to weight change in subjects participating in DIOGENES, weight loss and weight maintenance study performed in 8 centers across Europe for 8 months.

Methods: After an 8-week low calorie diet (LCD) subjects were randomized to 5 ad libitum diets for 6 months: high P/low GI (HP/LGI), high P/high GI (HP/HGI), low P/low GI (LP/LGI), low P/high GI (LP/HGI) and a control diet. Fatty acid composition in adipose lipids was assessed by gas chromatography in 261 subjects. Pearson correlations after transformation of data were calculated.

Results: Weight (W) and waist (WS) change after the weight management correlated significantly positively with initial percentage of palmitoleate (PO, 16:1n7) (only W), trans 16:1n-7, vaccenate (V, 18:1n-7), sum of trans 18:2n-6, sum of saturated FA, sum of trans FA and n-3 PUFA, negatively with linoleate (L, 18:2n-6) (only WS). When evaluating change in fatty acid percentage with change of weight and waist: positive correlation was shown with change in myristoleate (14:1n-5), PO (16:1n-7), V (18:1 n-7) (only W), L (18:2n-6) (only W), MEAD (20:3n-9) (only W), tFA (only W) and SCD1 (18:1n-9/18:0 and 16:1n-7/16:0). Negative correlations were found with change in stearate (18:0), oleate (18:1n-9) (only W) and eisosamonoenoate (20:1n-9).

Conclusion: The results suggest that higher decrease in myristeoleate and palmitoleate and SCD1 in adipose triglycerides related to higher decrease in weight and waist during weight management reflect decrease in endogenous lipogenesis.

Degradation pathway of sphingosine 1-phosphate preferentially mediates mast cell trafficking in the development of intestinal allergic responses

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Sphingosine 1-phosphate (S1P) is a lipid mediator regulating cell trafficking. We previously reported that pathogenic T and mast cells utilized S1P in their trafficking into the colon during the development of intestinal allergy. Thus, treatment with FTY720, an immunosuppresant to induce the down-regulation of S1P receptors, resulted in the reduced accumulation of pathogenic T and mast cells in the colon and consequent inhibition of intestinal allergy. In this study, we elucidated the function of S1P lyase, a key enzyme in the S1P degradation. To inhibit S1P lyase activity, we employed S1P lyase inhibors, 2-acetyl-5-tetrahydroxybutyl imidazole (THI) and 4-deoxypyridoxin (DOP). When mice were treated with eitheir THI or DOP, allergic diarrhea induced by oral inoculation of allergen was diminished. Like FTY720 treatment, THI or DOP treatment did not affect the allergen-specific serum IgE production, but inhibited mast cell trafficking into the colon. However, unlike FTY720, T cell migration into the colon was normally developed in mice receiving THI or DOP. These results suggest that the sensitivity to S1P lyase-mediated S1P metabolism is higher in mast cells than in T cells, and this pathway is a potential target in the control of intestinal allergy.

Competitive and metabolic steps of polyunsaturated fatty acids reflected in plasma phospholipids during fat substitution

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In essential fatty acid metabolism, alpha-linolenic acid (ALA, 18:3n-3) competes with linoleic acid (LA, 18:2n-6) for the Δ6-desaturase and inhibits LA conversion to arachidonic acid (AA, 20:4n-6), the precursor of prothrombotic and proinflammatory n-6 eicosanoids. The effects of AA can also be suppressed through ALA metabolism to eicosapentaenoic acid (EPA, 20:5n-3), antagonizing n-6 eicosanoid synthesis. However, ALA conversion to docosahexaenoic acid (DHA, 22:6n-3) can be interferred by low-ALA or high-LA diets or even cholesterol-lowering drugs, thus increasing AA/DHA ratio. Competitive effects and metabolism of ALA and LA in plasma phospholipids (PL) were compared in 148 subjects. Individual changes of AA/DHA ratio were monitored during 6-week fat substitutions. The data were taken from our previous studies [Seppänen-Laakso et al. 1992-93 (Study 1); 2001, 2010 (Study 2)]. Replacement covered 15-24% of fat intake, on average. Substitute fats were canola-type rapeseed oil (RSO), margarine (22% C18:1trans), olive and soyabean oils. At the baseline, individual AA/DHA values ranged between 0.88-6.32. When replacing butter by RSO (n=20; Study 1), the AA/DHA ratio (1.74) decreased (3 w, p=0.002; 6 w, p=0.0008). Instead of inhibiting AA, efficient ALA and LA metabolism to LC PUFA occurred. In subjects having low plasma fibrinogen levels (n=26; Study 2), the AA/DHA ratio (2.84) fell by RSO substitution (3 w, p=0.013; 6 w, p=0.01), due to the rise in DHA. When replacing low-ALA margarines by RSO (n=23, Study 1), ALA inhibited AA levels (p=0.01) and increased DHA slightly resulting in a decrease in AA/DHA (3 w, p=0.028). Replacement of butter by high-trans margarine (Study

1) or margarine by olive oil decreased AA/DHA ratio at 3 weeks. The results show that AA/DHA ratio in plasma PL can be lowered by RSO substitution. To suppress accumulation of excessive AA, effective inhibition by ALA and proper metabolism of ALA to DHA are required.

Impact of a single mixed Mediterranean-type meal compared to a high-fat meal on postprandial endothelial function and metabolic markers

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A high-saturated fat meal (HFM) has been shown to induce postprandial endothelial dysfunction, which is predictive of futur cardiovascular events but the postprandial effect of a single mixed Mediterranean-type meal (MMM) was never evaluated. Our objective was to evaluate postprandial endothelial and metabolic function in response to a MMM in comparison to an isocaloric HFM.

28 healthy non-smoking males have completed the research protocol. In random order on two separate days during a 1-week interval, subjects were fed two isocaloric meals after an overnight fast. The MMM (885 kcal) consisted of fresh salmon and vegetables baked in olive oil providing 51% of total calories from fat (7.87g SFA and 2.29g of omega-3). The HFM consisted of a McDonald's McMuffin and three hashbrowns (858 kcal) providing 58% of total energy from fat (14.78g SFA and no omega-3). Endothelial function was evaluated by measuring brachial artery flow-mediated dilation (%FMD) at baseline and at two (T2) and four (T4) hours postprandial.

Mean postprandial %FMD is less impaired following the MMM than the HFM (variation at T4 $-0.15\pm3.6\%$ vs $-2.83\pm3.3\%$ respectively, $p<0.05$). Postprandial increases of TG and TG/HDL at T4 were also less severe with the MMM than the HFM ($p\leq0.05$) and did not correlate to %FMD variations. When subdividing the population on the basis of the median fasting TG levels (0.90 mmol/L), the HFM led to significant endothelial impairment in the moderate-TG group while it had no effect in the low-TG group.

Our data suggest that a single MMM exerts less of a deleterious effect on postprandial endothelial function and metabolic markers than does a HFM. Moreover, subjects with higher fasting TG levels could be at higher risk of endothelial injury following a single HFM.

Data on postprandial oxydative stress and chylomicron content will be available in May 2012.

Effects of oleic acid on trophoblast amino acid uptake and mTOR signaling

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OBJECTIVE: The human placenta shows distinct changes in nutrient transport capacity in cases of altered fetal growth. For instance, activities of placental amino acid transporters are reduced in association with fetal growth restriction and increased with fetal overgrowth. However, the underlying mechanisms for altered nutrient transport in cases of pathological fetal growth are largely unknown. We have previously demonstrated that the mammalian target of rapamycin (mTOR) signaling pathway regulates placental amino acid transport. Because oleic acid has been reported to stimulate mTOR signaling in hepatocytes, we tested the hypothesis that oleic acid stimulates placental amino acid uptake and mTOR signaling.

METHODS: Cytotrophoblast cells were isolated from human, term placenta and cultured for 66 hours to allow for differentiation. The cells were cultured for a further 24 hours in presence or absence of 400 µM oleic acid. Activity of system A and system L amino acid transporters was measured using isotope-labeled tracers. mTOR signaling pathway activity was assessed by expression and phosphorylation of its downstream targets: eukaryotic initiation factor 4E-binding protein 1 (4EBP-1), ribosomal protein S6, and S6 kinase by western blot.

RESULTS: The activity of system A transporters was doubled after oleic acid treatment ($p<0.05$, t-test; $n=6$), while oleic acid had no effect on the system L transporter. Oleic acid treatment did not alter mTOR activation, as phosphorylation of 4EBP-1 (Thr37/46 and Thr70), ribosomal protein S6 (Ser235/236), and S6 kinase (Thr389) was not different between control and oleic acid treated trophoblast cells ($n=6$).

Similarly, oleic acid did not affect total expression levels of 4EBP-1, ribosomal protein S6, or S6 kinase (n=6).

CONCLUSION: Our data suggests that oleic acid stimulates placental amino acid transport through a mechanism independent of mTOR.

Identification of the Endocannabinoid Metabolome in Breast Milk at 2 Weeks of Lactation

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Endocannabinoids are endogenous cannabinoids that are lipid messengers and analogs of fatty acids. The endocannabinoid metabolome (ENDOMET) is associated with a variety of functions in central and peripheral physiology and pre- and postnatal development.   Evidence in mice pups suggests endocannabinoid regulation may underlie the initiation of the suckling reflex (Fride 2001, 2002, 2003). Using liquid chromatography-mass spectrometry, we quantified 13 plasma analytes of the ENDOMET in breast milk at two weeks postpartum from ten breast feeding women who had normal healthy pregnancies.   The regulation of these analytes are as follows [ng/ml: mean±SD, (range)]: anandamide, 0.078±0.05 (0.02-0.18); palmitoylethanolamine, 2.88 ±3.51 (0.7-12.47); oleoylethanolamine, 1.78±2.07 (0.45-7.33); docosahexaenoylethanolamine, 0.11±0.08 (0.04-0.31); 2-arachidonoylglyerol, 199±129 (12-471); 2-palmitoylglycerol, 26,830 ±22,362 (207-75,945); 2-oleoylglycerol, 4,550±3,625; 2-docosahexaenoylglycerol, 403 ±391 (14-1409); 2-eicosapentaenoylglycerol, (34±19 (7-59); 2-eicosapentanoylglycerol, 197 ±219 (33-790); arachidonic acid, 1,527 ±907 (348-3,079); docosahexaenoic acid, 765 ±553 (138-2,061); and eicosapentaenoic acid, 120 ±85 (12-280)]. These data provide evidence for the presence of these analytes in breast milk, which are also present in the plasma of pregnant women (Durham et al, 2010). These breast milk data point to a possible role of these metabolites in infant growth and /or development and this should be further explored.

Fatty Acids Quality, Developmental Nutrition and The Origins of Health or Disease in Adult Offspring

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Background: The mammalian fetus is completely dependent on the fatty acids supplied by its mother. Disturbances in nutrient supply in development can induce lasting alterations for growth and metabolism of the offspring throughout life. The implications of fatty acids quality in early origins of adult disease is still undisclosed .

Objective : In order to reveal the role of fatty acids quality in development and their impact on   health or disease in adult offspring, we generated a nutritional murine model.

Procedure : Female C57Bl6/J mice received experimental diets in pregnancy and lactation containing normal levels of oil rich in n-3/n-6 (RD), saturated (SFA), n-3 or n-6 essential fatty acids (EFA). The offspring were studied after 2 month RD and 2 month high fat diet (HFD:60% calories from fat) by analyzing metabolic syndrome parameters and fatty acid desaturases. Hepatic RNA was analyzed by microarrays.

Results : Although the body weight at birth and the growth curve were comparable, the adult's phenotype differed . SFA led to insulin resistance, body fat and tissue lipids higher than RD. Δ6 and Δ9 desaturase (SCD) were lower and higher ,respectively, than RD. n-3 ,conversely, led to the absence of metabolic syndrome, concomitantly with higher Δ6 and lower SCD activities than RD. n-6 led to results close to n-3 for fatty acid metabolism. Microarray analysis of n-3 showed up regulation of genes involved in lower food consumption ,higher energy expenditure and fatty acid oxidation and those of SFA showed up regulation of pathways leading to adipocyte development and higher SCD .

Conclusion : Our data clearly demonstrate that dietary fatty acids quality in development influences health or disease in adult offspring .The mechanisms seem to involve the ability of EFA n-3 to program genes for lower fat accumulation and SFA, in contrast, for fat development.

Omega-3 Fatty Acids and Traumatic Brain Injury

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Omega-3 fatty acids (n-3 FAs) have numerous proven benefits including support of cardiovascular and psychiatric health. Docosahexaenoic acid in particular, is found in high concentrations in the brain. N-3 FAs provide benefits by exerting a protective mechanism at the cellular and neuronal levels including the modulation of inflammatory cascade following brain injury. Promising preclinical research and evolving clinical experience now indicate that n-3 FAs are useful and effective for recovery following traumatic brain injury (TBI). Reported here is a case series of two dozen patients clinically managed following brain injury including two stroke patients and one severe TBI from a motor vehicle accident. With the exception of the severe TBI, all cases, mostly mild TBI, reported elimination of post concussive symptomatology within 48 hours using substantial doses of fish oil. The severe TBI case, in a permanent vegetative state, responded over a period of several months recovering most daily functionality. While not a drug or "cure" for brain injury, n-3 FAs are a powerful tool that can used to assist the brain to heal itself following injury.

Erythrocyte phospholipids fatty acids are associated with genetic variants in the FADS gene cluster in Chinese type 2 diabetes

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Background: The delta-5 and delta-6 desaturases, encoded by FADS1 and FADS2 genes, are key enzymes in polyunsaturated fatty acid (PUFA) metabolism. Single-nucleotide polymorphisms (SNPs) in FADS1 and FADS2 have been associated with the level of several long-chain n-3 and n-6 PUFA in blood phospholipids.

Objective: We explored the relation between FADS gene cluster polymorphisms and erythrocyte phospholipids (PL) fatty acids composition in a Chinese.

Methods: We have genotyped 3 SNPs located on the FADS1-FADS2-FADS3 gene cluster (chromosome 11q12-13.1) in the case-control study, which comprised 716 type 2 diabetes and 423 healthy subjects. Erythrocyte PL fatty acids were determined.

Results: FADS1 SNP (rs174537) was significantly associated with erythrocyte PL 20:3n-6 (p=0.050), 20:4n-6 (p for recessive model=0.046), 18:3n-6 (p for recessive model=0.042), n-3 PUFA (p for dominant model=0.050), n-3:n-6 (p for dominant model=0.048) in healthy subjects. FADS1 SNP (rs174537) was associated with erythrocyte PL 20:3n-6 (p for recessive model=0.043), 20:4n-6 (p=0.015), 22:6n-3 (p for recessive model=0.049) in type 2 diabetes. FADS2 SNP (rs174575) was associated with erythrocyte PL 20:4n-6 (p for dominant model =0.032), 22:5n-6 (p for dominant model =0.045), 18:2n-6 (p for dominant model =0.050) in healthy subjects. FADS2 SNP (rs174575) was associated with erythrocyte PL 18:2n-6 (p for recessive model=0.045) in type 2 diabetes. FADS3 (rs174455) was associated with erythrocyte PL 20:4n-6 (p=0.044), 18:3n-6 (p=0.025) in healthy subjects. FADS3 (rs174455) was associated with erythrocyte PL 20:4n-6 (p=0.010), 20:5n-3 (p=0.050), 22:4n-6 (p for recessive model =0.048), 22:5n-3 (p for recessive model =0.027) in type 2 diabetes.

Conclusions: FADS genotypes are associated with erythrocyte PL fatty acids composition in a Chinese.

Docosahexaenoic acid (DHA) supplementation during pregnancy increases the DHA content in human milk at early lactation

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Background: While postnatal DHA supports infant visual and cognitive development and increases DHA in human milk, the influence of prenatal supplementation on milk DHA is not well-described.

Objective: The objective of this study was to determine the effect of prenatal DHA supplementation on milk DHA.

Methods: Pregnant women (n=350) were enrolled in a phase 3 randomized-controlled clinical trial to evaluate the effects of prenatal DHA supplementation on infant development (NCT00266825). Women who lactated for at least 6 weeks (n=130) provided milk for analysis: 69 received 3 algal oil capsules (600 mg DHA total) and 61 received 3 soybean-corn oil capsules (control) per day, beginning before 20 weeks gestation and continuing until delivery. DHA (wt% total fatty acids) in maternal red blood cell phospholipid (RBC-PL) at delivery and in milk at 6 weeks postpartum was determined by gas liquid chromatography after transmethylation (boron trifluoride-methanol). Analysis of covariance was used to calculate the difference of DHA content in the milk between groups.

Results: Women in both groups consumed similar numbers of capsules per day (mean 2.6), corresponding to 523 mg DHA /day in the supplemented group. The DHA in maternal RBC-PL at delivery was higher in women who received DHA compared with the control group ($8.0 \pm 2.2\%$ vs. $4.9 \pm 1.3\%$, $p<0.001$). Milk from supplemented women had significantly higher DHA compared with the control group ($0.34 \pm 0.16\%$ vs. $0.24 \pm 0.13\%$, $p<0.001$).

Conclusion: Prenatal DHA supplementation increased mean DHA in milk at 6 weeks postpartum to the level shown previously in term formula-fed infants to enhance early visual and cognitive development. Supported by NIH (HD047315).

High Beta-Palmitate Fat Benefits the Intestinal Inflammatory Response and Reduces Intestinal Damage in Muc2 Deficient Mice

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Palmitic-acid presents 17-25% of human milk fatty acid content, with 70-75% of it attached to the sn-2 position of the glycerol backbone (beta-palmitate). Palmitic-acid in the sn-1 and sn-3 positions, the predominantly fat composition in regular infant formulas, is hydrolyzed by pancreatic lipase, resulting in free palmitic acid. The latter is poorly absorbed due to its high melting point and forms insoluble calcium soaps causing abdominal discomfort. In contrast, beta-palmitate structured triglyceride fat ingredient, with high levels of palmitic-acid in the sn-2 position, is well absorbed and mimics the fat composition and properties of human milk fat. The mucin MUC2 is the structural component of the intestinal mucus layer. Muc2 deficient (Muc2-/-) mice lack a protective mucus layer, and spontaneously develop severe colitis after weaning. The aim of the present study was to examine the potential protective role of high beta-palmitate fat (HBPF, InFat™, Advanced Lipids AB) in colitis development in Muc2-/- mice. Muc2-/- mice received 3 different synthetic diets: standard AIN-93G diet, diet with control fat or diet with HBPF (11.1%, 16.7% and 16.8% palmitic-acid, with 6.3% 11.0% and 50.4% palmitic-acid at the sn-2 position respectively), for a period of 5 weeks after weaning. Clinical symptoms, intestinal morphology, and inflammation in the distal colon were analyzed. HBPF reduced the extent of intestinal erosions and limited the intestinal morphological damage. Pro-inflammatory cytokines did not differ among the three diet groups. However, the pro-inflammatory response in the HBPF group was counterbalanced by an immunosuppressive response, which was demonstrated by increased mRNA expression of Foxp3, Il10, Il12a, Ebi3 and Pparg. In conclusion, this study shows for the first time that high beta-palmitate diet limits intestinal mucosal damage and controls the intestinal inflammatory response in Muc2-/- mice.

Microwave irradiation accelerated fatty acid analysis and its application in finger-pricked whole blood samples of deploying soldiers

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To evaluate the essential fatty acid status in finger-pricked whole blood samples from deploying soldiers, a microwave irradiation accelerated fatty acid analysis was developed from Lepage & Roy's (L&R) direct one-step transesterification with methanol catalyzed by acetyl chloride. One explosive-proof multimode microwave synthesis system (named as MARS) was employed for derivatization reaction. Finger-pricked

blood samples collected on filter paper from pre-deployed soldiers (n = 191) were placed in safety-proofed glass vessel containing a mixture of methanol, hexane, acetyl chloride and internal standard. The vessels were then heated in MARS at 125°C for 5 min with power of 1600 W. Afterwards, neutralized by an addition of sodium carbonate solution followed by centrifugation. One aliquot of the upper phase was injected into 7890A GC system coupled with a high efficient DB-FFAP capillary column. Compared to the fatty acid concentration measured by conventional L&R, the fatty acids from microwave assay were ≥ 94% in human plasma, ≥ 93% in whole blood on filter paper for all identified fatty acids except 80% (plasma), 72% (whole blood) for the usually minor fatty acids 20:0, 22:0, 24:0 and 24:1n-9. The degradation of polyunsaturates was observed in whole blood sample collected on filter paper pre-treated with antioxidant. The fatty acid composition (wt%, mean ± SD) in deploying soldiers for linoleic acid was 19.9 ± 2.9%; arachidonic acid was 8.2 ± 1.6%; linolenic acid was 0.47 ± 0.16%; and docosahexaenoic acid was 1.40 ± 0.41%. The proportion of omega-6 in total highly unsaturated fatty acid was 83.1 ± 3.1%, omega-3 was 16.9 ± 3.1%. This microwave accelerated fatty acid analysis could be applied in both the absolute quantification (mg/L) and relative quantification (weight%) of fatty acids in human finger-pricked whole blood on filter paper.

A new blood spot method for determining the fatty acid status of individuals: contamination and oxidation

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There have been many reports of the use of blood spot technology to evaluate the fatty acid status in human subjects. Two problems were found from current blood spot method, namely, contamination during sample processing and oxidation during sample storage. The contaminant problem was resolved by careful selection of collection papers, gloves, and storage bags that were shown to contain the minimum amount of contaminants. The oxidation problem was resolved by using a combination of protectants and a special blood collection matrix, and this treatment combination retained more than 90% of the original EPA and DHA content in the blood spot following 2 months of storage at room temperature. This represented a significant improvement in stability compared with previously reported standard blood spot protection systems using BHT as antioxidant and Fluka test kit paper as a collection paper that only retained ~60% of the n-3 LCPUFA content in the applied blood spots over the same time period. This is the first report of a protection system capable of stabilizing the LCPUFA in human blood spot samples for extended periods when stored at room temperature which could have important applications for clinical studies and large-scale diagnostic screening in humans.

Motor development is positively related to DHA status in breastfed African and Dutch infants

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Background. Docosahexaenoic (DHA) and arachidonic (AA), acids are important for neurodevelopment. We investigated the relation between erythrocyte (RBC) DHA and AA contents and neurological development, by assessment of General Movements (GMs), in populations with substantial differences in fish intakes.

Methods. We included 3 months old breastfed infants of 3 Tanzanian tribes; Maasai (low fish n=5); Pare (intermediate fish n=32); Sengerema (high fish n=60) and a Dutch population (low-intermediate fish n=15). GMs were assessed by motor optimality score (MOS) and the number of observed movement patterns (OMP; a MOS sub score). RBC-DHA and AA contents were determined by capillary gas chromatography.

Results. There were no between-population differences in MOS. OMP of Sengerema infants (high fish) was higher than OMP of Dutch infants (low-intermediate fish). OMP related positively to infant age (p<0.001) and RBC-DHA (p=0.011), and was unrelated to ethnicity and RBC-AA.

Conclusion. Movement quality of 3 months old infants is positively related to stable DHA status.

Effect of n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation in pregnancy on infant allergies in the first year of life: a randomised controlled trial

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Objective: To determine whether dietary n-3 long chain polyunsaturated fatty acid (LCPUFA) supplementation of pregnant women with a fetus at high-risk of allergic disease will reduce immunoglobulin E-associated eczema or food allergy at one year of age.

Design: Follow-up of 706 infants at high-hereditary risk of developing allergic disease whose mothers were participating in the Docosahexaenoic Acid to Optimize Mother Infant Outcome (DOMInO) trial. The intervention group (n= 368) were randomly allocated to receive fish oil capsules (providing 900mg n-3 LCPUFA daily) while the control group (n= 338) received matched vegetable oil capsules without n-3 LCPUFA from 21 weeks gestation until birth. The primary outcome was immunoglobulin E-associated allergic disease (eczema or food allergy with sensitization) at one year of age.

Results: There were no differences in the overall percentage of infants with immunoglobulin E-associated allergic disease between the n-3 LCPUFA and control groups (32/368 (8.6%) vs 43/338 (12.7%); adjusted relative risk 0.70; 95% CI 0.45 to 1.09; P=0.12), although the percentage of infants diagnosed with atopic eczema (i.e eczema with associated sensitization) was lower in the n-3 LCPUFA group (26/368 (7.1%) n-3 LCPUFA vs 39/338 (11.7%) control; unadjusted relative risk, 0.61; 95% CI 0.38 to 0.98; P=0.04; adjusted relative risk, 0.64; 95% CI 0.40 to 1.02; P=0.06). Fewer infants were sensitized to egg in the n-3 LCPUFA group (34/368 (9.3%) vs 52/338 (15.4%) control; adjusted relative risk, 0.62; 95% CI 0.41 to 0.93; P=0.02), but there was no difference in immunoglobulin E-associated food allergy between groups.

Conclusion: n-3 LCPUFA supplementation in pregnancy did not reduce the overall incidence of immunoglobulin E-associated allergies in the first year of life, although atopic eczema and egg sensitization were lower. Longer term follow-up is required to determine if there is an effect on respiratory allergic diseases and aeroallergen sensitization in childhood.

Effects of iron and n-3 fatty acid supplementation on the fatty acid composition of peripheral blood mononuclear cells and absenteeism in iron-deficient primary school children in South Africa

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Background: n-3 long-chain polyunsaturated fatty acids (LCPUFA) are essential for proper membrane composition and functioning of cells, including peripheral blood mononuclear cells (PBMC).

Objectives: This trial investigated the effects of iron and n-3 LCPUFA supplementation, alone and in combination, on PBMC membrane FA composition and absenteeism.

Study design: In a 2x2 factorial, double-blind, controlled trial, subjects (n=321) were randomly assigned to one of four groups receiving 1) docosahexaenoic/eicosapentaenoic acid (DHA/EPA, 420mg/80mg) + iron (50mg as ferrous sulphate); 2) DHA/EPA + placebo; 3) placebo + iron; or 4) placebo + placebo for 8.5 months. Absenteeism and health was monitored daily. Biochemical indicators and PBMC total phospholipid FA composition (including trans FA) were measured at baseline and endpoint in a sub-sample (n=156). Hepcidin concentrations were measured in 53 subjects at baseline.

Results: DHA/EPA supplementation significantly increased EPA (estimated effect size: 0.10, 95%CI: 0.05, 0.14) and DHA (0.63, 95%CI: 0.43, 0.83), and decreased the relative composition of total n-6 LCPUFA (-1.56, 95% CI: -3.77, 0.65) in PBMC. Interestingly, there was a significant iron treatment effect to lower trans FA composition (-0.19, 95%CI: -0.37, -0.004). There was no significant treatment effect of iron on n-3 LCPUFA in PBMC. However, in linear regression analysis excluding subjects receiving DHA/EPA, body iron stores was a significant predictor of DHA (β=0.234, 0.042) and EPA (β=0.263,

P=0.034) at endpoint, controlling for age, gender, school and respective baseline DHA. At baseline, EPA in PBMC membranes tended to be a significant predictor of hepcidin (β= -0.253, P=0.054). Data on absenteeism will be reported at the congress.

Conclusion: Our results suggest that n-3 LCPUFA supplementation in iron-deficient children is effective in improving relative composition of n-3 LCPUFA in PBMC at the expense of n-6 LCPUFA. Furthermore, this is the first study to show an effect of iron supplementation on trans FA composition in membranes.

Polymodal dose-effect of alfa-tocopherol on the lipid structure of cell membranes in vitro

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Alpha-tocopherol (TL) or vitamin E is an effective natural antioxidant, which plays important role in cell regulation as inhibitor of lipid oxidation and structural factor in biological membranes. In the present work it was studied the effect of TL on the cell membranes in a wide range of concentration: from high (equivalent to physiological) to ultra-low concentrations (ULC<10 in -12 degree mol/l) on the structural parameters of membrane lipids in vitro. The plasmatic (PM) and endoplasmic reticulum membranes (ERM) were isolated from liver cells of mice. The dynamic lipid structure studied by EPR-technique on the computerized spectrometer Bruker-EMX using two spin-probes: 5- and 16-doxylstearic acids (5- and 16-DSA). The nonlinear and polymodal "dose-effect" dependencies of microviscosity value estimated by τ_c of 16-DSA and order parameters S of 5-DSA have been found. It was observed three "waves" of microviscosity increase: at concentration 10 in -4 degree -10 in -9 degree mol/l explained by incorporation of TL into the membrane lipids; within ULC range of TL (10 in -9 degree -10 in -18degree mol/l) it is conditioned by formation of the micro-domains and rafts or changes of them induced by TL; at lower than 10 in -18 degree mol/l (ultra-low dilutions - ULD) of TL it can be related with water structure of solutions. It was shown an increase of parameter S at high concentration of TL and extremely at ULC of TL in both membranes (significantly in PM), which correlated with activity of protein kinase C. It was shown that the effect of TL on membrane lipid structure depends on polarity of solvent. The thermo-induced structural transitions (TST) studied in PM and ERM showed an appearance of additional TST at the range of physiological temperature (307-314K) upon the effect of different concentration of TL on the membranes. It was found a correlation between lipid structural parameters and properties and diameter of nano-associates formed in solution of TL. It was concluded that polymodal effect of TL in a wide range of concentration on cell membranes is typical to "dose-effect "of the drugs affecting on cells at ULC and can be important for membrane regulation.

Green tea catechins attenuate the production of 12-HETE in human skin following exposure to ultraviolet radiation

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The sunburn inflammatory response is partly mediated by upregulation of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes. Green tea catechins (GTC) exhibit potent anti-inflammatory properties, and thus may offer systemic photoprotection. Here, we report the inhibitory effect of GTC on 12-hydroxyeicosatetrenoic acid (12-HETE), a pro-inflammatory chemo-attractant 12-LOX-derived metabolite that is found upregulated in human skin following exposure to UVR.

Participants (n=14, 27-56 yrs, all female, phototype I/II) were supplemented with GTC (550 mg/day) with vitamin C (50 mg/day; stabiliser), for 12 weeks. Minimal erythema dose (MED) was determined pre- and post- supplementation using solar simulated radiation (SSR). Buttock skin was irradiated with 3x MED, after 24h skin punch biopsies and suction blister fluid were collected. Urine samples were collected to determine compliance.

Lipidomic analysis of suction blister fluid by liquid chromatography electrospray ionisation tandem mass spectrometry (LC/ESI-MS/MS) revealed that in basal un-irradiated skin the dominant eicosanoids were

prostaglandin (PG) E2 and 12-HETE (49.1 pg/μL ± 11.5 and 13.3 pg/ μL ± 2.2, respectively). Following UV irradiation, both PGE2 and 12-HETE were significantly up-regulated to 115.3 pg/ μL ± 17.2 and 64.4 pg/ μL ± 11.8 respectively (p<0.05). Following supplementation, a significant reduction in the production of UVR-induced 12-HETE was observed (41.2 pg/ μL ± 8.4, p=0.015 compared to basal UV-irradiated skin; paired samples T test). However, no significant changes were observed for UVR-induced PGE2 levels.

Furthermore, a significant reduction was observed in skin erythema at higher UVR doses (p<0.05) post-supplementation, whilst LC/ESI-MS/MS analysis identified an increased number of catechin metabolites in skin biopsies. Finally, good compliance with GTC supplementation was confirmed with identification of major GTC species in urine.

The results presented here indicate that GTC attenuate the UVR-induced production of 12-HETE and, consequently, may affect the degree of sunburn inflammation, suggesting a potential use for GTC as systemic photoprotective agents.

Attenuating posttraumatic stress symptoms with omega-3 polyunsaturated fatty acids among rescue workers after the Great East Japan Earthquake: Feasibility of a filed randomized controlled trial

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Background: On March 11, 2011, the Great East Japan Earthquake left about 20,000 dead or missing. Mobile medical rescue workers (physician, nurses, operational coordination staff) were dispatched to areas with large-scale destruction and multiple injured and sick casualties. Previous studies have pointed out posttraumatic stress disorder (PTSD) among rescue workers as well as the need for screening and prevention for PTSD. So far we have shown in an open trial that PTSD symptoms in critically injured patients can be reduced by taking omega-3 fatty acids intended to stimulate hippocampal neurogenesis. There was no field clinical trial of omega-3 fatty acids for preventing PTSD.

Method: This study was designed to determine the effectiveness of attenuating PTSD symptoms with omega-3 fatty acids among rescue workers after the disaster. First, we provided psycho-education on PTSD symptoms, which was common in responders to rescue workers deployed to the disaster area. Second, observational study was conducted to evaluate PTSD symptoms at 1 month after the diaster. Third, rescue workers who provide consent to participate in the intervention research were randomly divided into a group given an omega-3 fatty acid supplement and a group not given the supplements. The required sample size for intervention research was estimated at 48 cases per group. We assessed PTSD symptoms by the Impact of Event Scale revised at 4 months after the disaster.

Results: Of the 1,816 rescue workers, 426 from all over Japan participated in the observational study. Of 426, 172 agreed to participate in clinical trial.

Discussion: One fourth of rescue workers responded observational study, however only 9% participated in clinical trial, which could limit the external validity. From a viewpoint of study design, sample size was enough to assess the effectiveness of omega-3 fatty acids for preventing PTSD. This study showed that acute intervention after the disaster with omega-3 fatty acids would be feasible.

Associations between maternal PUFA status, MeHg exposure and telomere length in Seychellois mothers and children

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Background: Short mean telomere length (TL) in peripheral blood is associated with increased oxidative stress. Dietary n-3 PUFA has been shown to exert protective effects against TL shortening in adults. It is unknown whether PUFA status during pregnancy could influence TL of the mother or child and whether

co-exposure to the neurotoxin methyl mercury (MeHg), from fish consumption, could modify this association.

Objective: To examine the association between maternal PUFA status, maternal and child TL and to investigate the potential confounding effect of MeHg in a high fish eating population.

Procedure: Subjects were mother-child pairs (n=272) enrolled in the Seychelles Child Development Study Nutrition Cohort 1. Relative mean leukocyte TL was measured by quantitative polymerase chain reaction in blood samples available from mothers at 28 weeks gestation and their children aged 5 years. Previously we measured total Hg in maternal hair samples (proxy for MeHg exposure) and total serum PUFA in maternal blood at 28 wks gestation and delivery. Linear regression models were run for total n-3 and n-6 PUFA, and separately for n-6: n-3 using the geometric mean of the two PUFA values. All models were adjusted for maternal hair MeHg and for factors known to influence TL in adults, including socioeconomic status (SES), age, BMI, smoking, alcohol and in children; sex, birthweight, BMI and PROCESS.

Results: Mean relative TL was 0.64 + 0.11 and 0.71 + 0.10 in mothers and children respectively. Maternal n-6: n-3 was a significant positive predictor of maternal TL (β= 0.001, p=0.048) indicating a beneficial influence. At 5 years of age, neither the mothers' PUFA status nor MeHg exposure were associated with child TL.

Conclusion: Our preliminary findings suggest that maternal PUFA status may influence maternal but not child TL. Further investigation is needed to elucidate differential effects of n-3 and n-6 PUFA on average TL.

Time course of lipid composition changes in blood and skeletal muscle during a 4 week period of fish oil ingestion in humans

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Increased human skeletal muscle n-3 phospholipid content is suggested to modulate skeletal muscle responsiveness to nutrients. However, data on incorporation of ingested n-3 fatty acids into human skeletal muscle are limited. We aimed to characterise the time-course of n-3 incorporation into skeletal muscle alongside changes in blood lipid composition during oral fish-oil supplementation. Ten healthy male participants (aged 21±3 yrs; mass 76±5kg) consumed 6g/d of fish oil capsules for 4wks. Muscle biopsies and fasted venous blood samples were obtained 2 weeks prior, and at 0, 1, 2 and 4 wks of fish oil supplementation for assessment of lipid composition changes. Lipid composition of muscle and blood cell membrane was assessed by GC-FID. Lipid composition of blood and muscle membranes were not different between -2 and 0 wks. An increase in %total n-3 PUFA in muscle was observed at 2wks and continued to rise at 4wks (P<0.05). Blood %total n-3 increased by 1wk (P<0.05) and remained elevated for the remaining timepoints. There was no difference over time (0-4wks) for %total n-6 in muscle (36.4±2.7 to 35.1±1.8%) with a small but significant reduction between 0 and 4wks in blood (33.5±1.9 to 29.4±1.4%). The 20:4n-6/20:5n-3 ratio in muscle was lower by 1wk and declined further at 4wks (P<0.05). A decline in 20:4n-6/20:5n-3 ratio in blood was observed by 1wk (P<0.05) with no further reduction at 4wks. %n-3/total HUFA in muscle increased from 0 to 4wks (21.4± 5.5 to 34.7± 6.7%, P<0.05), but in blood this increased from 0 to 1wk (26.2±4.4 to 37.8±4.7%), and was further elevated at 4wks (to 45.6± 5.0%, P<0.05). These data indicate a slower timecourse of n-3 incorporation into muscle than blood, likely reflecting tissue turnover times. Longer periods of supplementation are required to achieve stable n-3 content in skeletal muscle tissue at the ingested dose examined in this study.

Post-prandial effects of a meal rich in long-chain omega-3 fatty acids on indicators of cardiovascular risk

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Based on epidemiological evidence of their long-term beneficial effects on the risk of cardiovascular disease, the current UK dietary recommendation for fish consumption provides 0.45g long-chain omega-3

fatty acids (LC n-3 PUFA) per day. Less is known about the immediate post-prandial effects, however, therefore this pilot project aimed to investigate post-prandial responses in markers of cardiovascular risk to a test meal naturally rich in LC n-3 PUFA compared with a control meal.

On different days, participants randomly received one of two meals (970 kcal, 48% fat) differing only in their LC n-3 PUFA content: a test meal (salmon, 9.7g total LC n-3 PUFA),or a control meal (ham, <0.2g total LC n-3 PUFA). Habitual LC n-3 PUFA intake was assessed by food frequency questionnaire. Measurements of arterial compliance (pulse wave velocity, PWV; augmentation index, AIx), whole blood fatty acids, plasma glucose, plasma insulin, markers of endothelial dysfunction (MCP-1, NO-derived nitrites), and oxidative stress (NF-κB, sE-selectin, tBARS) were assessed at baseline and at intervals for 4 hours post-prandially.

Five healthy males (age 26±4 yrs) at low risk of cardiovascular disease (BMI 22.4±1.0 kg/m2, waist circumference 77.3±4.4 cm) were recruited. The mean habitual intake of LC n-3 PUFA was 0.57±0.47g/day. There was no difference in PWV over time between the test and control meals (p=0.21), however over time the AIx for the control meal decreased compared with the test meal, which remained stable (p=0.014). Whole blood EPA levels increased significantly over the 4 hour post-prandial period for the test meal compared with the control meal (p=0.002).

Dietary intake of a meal rich in LC n-3 PUFA results in an increase in post-prandial EPA levels, and reduces the decline in AIx seen with the control meal. Once the remaining data is available, it will provide additional insight into the mechanism of these responses.

Omega-3 Fatty Acid Deficiency During Development Lead to Structural Alterations in the Adult Rat Limbic Forebrain: An in vivo Magnetic Resonance Imaging Study

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Background: Prior evidence suggests that the primary long-chain omega-3 fatty acid found in brain, docosahexaenoic acid (DHA), has neurotrophic and neuroprotective properties, and that deficits in brain DHA accrual during adolescent development lead to elevated behavioral indices of depression and aggression in adult rats. Moreover, mood disorders associated with long-chain omega-3 fatty acid deficits including bipolar disorder, frequently initiate during childhood and adolescence and are associated with structural abnormalities in prefrontal and limbic brain regions.

Objective: To determine the effect of omega-3 fatty acid deficiency during adolescent development on adult rat brain structure by in vivo magnetic resonance imaging.

Procedure: A post-weaning (P21-P90) dietary alpha-linolenic acid (ALA, 18:3n-3) depletion model was used. In adulthood, controls (ALA+) (n=10) and post-weaning (n=10) ALA- rats were anesthetized and scanned in a 7T Bruker Biospec system. Relative group differences in regional structural volumes were determined using a voxel-based morphometry analysis and corrected for multiple comparisons. Postmortem cortical DHA composition was determined by gas chromatography.

Results: Compared with controls, cortical docosahexaenoic acid (DHA, 22:6n-3) composition was significantly reduced in adult post-weaning ALA- rats (-28%, p<0.0001). Compared with controls, post-weaning ALA- rats exhibited increases in volume in several limbic forebrain structures including the right olfactory tubercle, bilateral insular cortex, bilateral nucleus accumbens, and right amygdala. There were no significant group differences in hindbrain structures including the cerebellum.

Conclusion: These preclinical imaging data demonstrate that moderate reductions in brain DHA accretion during adolescence lead to robust structural alterations in the adult rat limbic forebrain. It is notable that these brain regions are thought to regulate motivational and emotional processes, and have repeatedly been implicated in the pathophysiology of mood disorders.

Adolescents with Major Depressive Disorder Exhibit Reversible Erythrocyte Omega-3 Fatty Acid Deficits: Associations with Mood Symptoms and Functional Cortical Activity

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Background: We previously reported that supplementation with docosahexaenoic acid (DHA, 22:6n-3), the principal long-chain omega-3 (n-3) fatty acid in brain, increased functional cortical activity in healthy children by magnetic resonance imaging (fMRI). Although evidence suggests that major depressive disorder (MDD) is associated with peripheral and central DHA deficits, there is currently little known about the relationship between DHA status, functional cortical activity, and mood symptoms in MDD patients.

Objective: To determine the relationship between long-chain n-3 fatty acid status, mood symptoms, and functional cortical activity in adolescent MDD patients.

Procedure: Erythrocyte fatty acid composition was determined in adolescents (10-20 years) meeting DSM-IV criteria for MDD (n=20) and healthy adolescents (n=20). Patients were then randomized to one of two doses of fish oil (2.4 or 15 g/d) for 10 weeks. At baseline and endpoint, depression symptom severity (CDRS-R) and functional cortical activity during performance of a sustained attention task (CPT-IP) were determined by fMRI.

Results: Adolescents with MDD exhibited significantly lower erythrocyte DHA levels compared with healthy adolescents (-26%, p=0.004). At baseline, erythrocyte DHA was positively correlated with functional activation in the superior frontal gyrus (BA10), medial frontal gyrus (BA9), and anterior cingulate (BA23). Fish oil supplementation significantly increased erythrocyte DHA composition in low-dose (+49%, p<0.0001) and high-dose (+52%, p=0.0001) groups at 10 weeks. Baseline CDRS total scores declined significantly in low-dose (-18%, p=0.01) and high-dose (-38%, p<0.0001) groups at 10 weeks. However, there were no significant baseline-endpoint changes in functional activity in low- or high-dose groups. At 10 weeks, erythrocyte DHA was now positively correlated with activation of the parahippocampal gyrus.

Conclusion: Adolescent MDD is associated with erythrocyte DHA deficits which are correlated with functional activity in the prefrontal cortex. Dietary-induced elevations in erythrocyte DHA levels are associated with reductions in depression symptom severity independent of changes in functional cortical activity.

Maternal Lipid and Fatty Acid Changes in Early Pregnancy

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Background: Maternal fatty acids are mobilised by 13 weeks' gestation, but data earlier in pregnancy is lacking. Objective: To assess changes in maternal lipid and fatty acid metabolism at <8 weeks' gestation. Procedure: Fasting samples were collected from women undergoing a natural cycle frozen embryo transfer (FET) at the lutenising hormone surge (day 0), at FET (day 3, non-fasting) and on days 7, 10, 18, 29 and 45. Repeated measures ANOVA with post hoc Tukey's test was used to identify parameters which changed over time. Results: Of 161 women recruited, 38 women had a successful pregnancy (positive fetal heartbeat at day 45) and 123 failed to result in pregnancy. The earliest lipid change was day 10 when total cholesterol was decreased by 8% (p=0.04) and further reduced to 10% (p<0.001) lower than baseline by day 18-45. By day 18 plasma triglycerides (20%) and HDL-C (7%) levels were decreased (p<0.001) but rebounded by day 45. By day 18 plasma and erythrocyte concentrations of linoleic acid decreased by 10% (p<0.001) whereas delta-6 desaturase activity (28%, p<0.005), long chain (LC) PUFA (9%,p<0.005) and erythrocyte nervonic acid (6%, p<0.005) increased. By day 29 DHA increased by 29% (p<0.005) and gamma-linolenic acid (27%, p<0.005) and elongase activity (10%, p<0.005) decreased. By day 45, n-6 PUFA were increased by up to 54%, (p<0.005); stearoyl CoA desaturase activity increased by 11% (p<0.005); and erythrocyte EPA decreased by 20% (p<0.005). Conclusion: Changes in maternal

lipids were observed as early as 10 days' gestation. By day 18, when the embryo has a very high demand for membrane formation, the changes indicated mobilisation and synthesis of both n-6 and n-3 LC PUFA. The specific mobilisation of DHA and nervonic acid both essential for brain development are also key early events.

A multi-nutrient composition with neuroprotective properties in spinal cord injury

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Spinal cord injury (SCI) leads to a significantly reduced quality of life for patients. There is an unmet clinical need for agents which protect and repair the injured spinal cord. SCI leads to tissue loss, due to a multitude of mechanisms activated by the initial event. The delayed injury phase involves mechanisms including excitotoxicity, increased inflammation and oxidative stress. The protracted changes and the degeneration caused by secondary injury continue in the following days and weeks after trauma. Therapeutic strategies in SCI aim to limit the impact of the secondary injury and also help the regeneration of the tissue. We have shown that i.v. injected omega-3 fatty acids such as docosahexaenoic acid have beneficial effects after SCI in rats. The aim of the present project was to test an oral multi-nutrient preparation containing omega-3 fatty acids and other nutrients. The nutrients in this preparation have previously been shown to act synergistically to increase membrane formation and are suggested to have therapeutic potential in Alzheimer's disease. Adult rats received a compression SCI at thoracic level and were fed a control diet or a multi-nutrient fortified diet for nine weeks following injury. The neurological function of the animals was assessed using the BBB locomotor score and subscore, and at the end of the treatment the animals were sacrificed for tissue analysis. The supplementation of the diet with multi-nutrients led to a significantly better locomotor recovery. It also increased neuronal and glial survival and improved markers of cytoskeletal integrity. The tissue of the animals treated with the multi-nutrient concept also showed a significantly reduced inflammatory microglia and macrophage response. These results suggest that the combination of nutrients has significant therapeutic potential in SCI. To our knowledge this is the first orally administered multi-nutrient containing preparation which is showing promise in spinal cord trauma.

Peripheral nerve injury has a significantly improved outcome in fat-1 mice

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Peripheral nerve injury (PNI) often leads to an unsatisfactory neurological outcome and a partial recovery of function. There is a need for therapies that protect peripheral neurons against injury and enhance regeneration. Long-chain omega-3 polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid have been shown to have therapeutic potential in acute traumatic injury in the central nervous system. In the present study we explored their potential in PNI. We investigated this in mice which express the fat-1 gene, which leads to an increase in endogenous omega-3 PUFAs and a concomitant decrease in the omega-6/omega-3 PUFA ratio. Dorsal root ganglion (DRG) primary sensory neurons from wild-type or fat-1 mice were subjected in vitro to a mechanical strain or hypoxic injury and cell death was assessed using ethidium homodimer-1 labelling. The fat-1 background appeared to confer significant neuroprotection against both injuries. We then examined the impact of the fat-1 background on PNI by assessing early functional and morphological changes in wild-type and fat-1 mice after a sciatic nerve crush. At 7 days after injury, an accelerated functional recovery post-injury was seen in fat-1 mice compared to the wild-

type controls, when assessed using von Frey filaments for mechanical stimulation threshold and the sciatic nerve functional index. These observations were then correlated with histological injury-related markers. The injury-induced expression of the factor ATF-3 was decreased in the DRG of fat-1 mice. In contrast, the axons detected distal to the crush region, were increased in the transgenic animals. Fat-1 animals also had some protection against the muscle atrophy which occurred following injury. In conclusion, both the in vitro and in vivo data provide support to the idea that a higher endogenous omega-3 PUFA level could lead to a less damaging impact of injuries to peripheral nerves.

Detailed characterization of gangliosides in prostate cells using LC/MS

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Gangliosides influence a number of tumour cell properties including growth, signaling, and differentiation. Characterizing ganglioside profile in tumour tissues may improve disease prognosis and allow for improved survival. Novel biomarker panels for prostate cancer are required to determine which tumours will develop into aggressive disease. Low abundance and heterogenous nature of gangliosides makes detection in biological samples challenging. Liquid chromatography (LC) hyphenated with mass spectrometry (MS) is an emerging technique in lipid analysis due to its high sensitivity, speed, and specificity. Two malignant prostate cell lines (PC-3, LNCaP) and one cell line representing healthy prostate (RWPE-1) were analysed with LC/MS to compare respective ganglioside profiles. Cells were subjected to a Folch extraction to isolate the gangliosides. Extracts were injected onto a C18 column where the gangliosides were separated by reverse-phase chromatography prior to detection on an Agilent 6430 triple-Quad LC/MS operated in multiple reaction monitoring mode. The cells were screened against a database of 250 mono- and disialylated gangliosides. GD1 was the most abundant ganglioside class and exhibited a high degree of heterogeneity within the ceramide moiety. The ceramide consisted of sphingosine (d18:1) or dihydrosphingosine (d18:0); and fatty acid chains of C16:0, C22:0, C24:0, C24:1, and C26:0 were observed. Interestingly, LNCaP had a relatively low signal for GD1 species compared to RWPE-1 and PC-3. In contrast to the GD1 profile, GD3 and GM3 were observed in PC-3 and RWPE-1 cells with only one predominate ceramide composition (d18:1/C16:0). RWPE-1 cells had an increased abundance of GD3 species relative to GM3; the trend was reversed in PC-3 cells. These findings warrant further investigation into ganglioside profiling as a clinical tool for assessing invasiveness of prostate cancer.

Improvements in childhood learning and behaviour accompany increases in omega-3 status

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Background – Previous studies indicate that supplementation with the omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) may improve symptoms in children with ADHD. However, the relative benefits of these fatty acids are unknown.

Objective – To compare effects of supplementation with DHA-rich and EPA-rich oils versus safflower oil on cognition, literacy and behaviour in children with ADHD symptoms.

Design – Ninety children were recruited for a 12-month double-blind placebo-controlled three-way crossover trial. Supplements high in EPA (1109 mg EPA + 108 mg DHA/day), DHA (264 mg EPA + 1032 mg DHA/day) and linoleic acid (safflower oil, 1467 mg LA/day) were consumed in random order for four months each. Erythrocyte fatty acids, assessments of attention, cognition and literacy and Conner's Parent Rating Scales of behaviour were measured at 0, 4, 8 and 12 months.

Outcomes – Fifty three volunteers completed the trial. There were no significant differences in outcome measures between the 3 treatment phases. However, in children with erythrocyte fatty acid data obtained at the end of each treatment phase (n=76-46), within-subject increases in EPA+DHA were associated with improvements in spelling (r=.365, B=0.540, p<.001), attention (r=-.540, B=-0.429, p<.001), and parent ratings of oppositional behaviour (r=-.301, B=-0.517, p.003), hyperactive behaviour (r=-.310, B=-

0.509, p<.001), cognitive problems (r=-.326, B=-0.737, p<.001), DSM-IV hyperactive symptoms (r=-.270, B=-.428, p=.002) and DSM-IV inattentive symptoms (r=-.343, B=-0.647, p<.001) on the CPRS. Increased n-3 PUFA, EPA, DHA and decreased n-6 PUFA and n-6/n-3 PUFA ratio were also associated with improvements within subjects after supplementation; however, the combination of EPA+DHA (i.e. the Omega-3 Index) and also n-6/n-3 PUFA ratio gave the most consistent correlations.

Conclusion – Increasing erythrocyte DHA and EPA via increased dietary intake of n-3 PUFA may improve behaviour, attention and literacy in children with ADHD symptoms. Both fatty acids appear to be beneficial.

The leptin receptor Gln223Arg polymorphism (rs1137101) mediates the postprandial lipaemic response in adult males

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The role of leptin in systemic macronutrient metabolism is being increasingly recognised. Our aim was to examine the impact of the common leptin receptor (LEPR) Gln223Arg polymorphism (rs1137101) on postprandial lipaemia. A total of 231 adults, 122 males (mean age 52.3 ± 0.9 y and BMI 27.1 ± 0.3 kg/m2) and 109 females (mean age 52.2 ± 1.1 y and BMI 25.4 ± 0.3 kg/m2), underwent a sequential meal postprandial investigation (480 min), in which 10 blood samples were taken after a test breakfast (t=0 min, 49 g fat) and lunch (t=330 min, 29 g fat). Fasting total- and low-density lipoprotein cholesterol were lower in the ArgArg than GlnArg group (P<0.04), whereas fasting TAG was lower in the ArgArg than GlnGln group (P<0.02). Area under the curve (AUC) and incremental AUC for the postprandial TAG response were lower in the ArgArg than GlnGln and GlnArg individuals (P≤0.023, TG AUC (mmol/l x 480 min) of 1227, 1063 and 904 in GlnGln, GlnArg, and ArgArg, respectively). Genotype*gender interactions were evident for fasting and postprandial TAG responses (P<0.05), with the genotype effect only evident in males. Regression analysis indicated that the LEPR genotype and genotype*sex interactions were independent predictors of the TAG AUC, accounting for 6.3% of the variance. Our main findings were replicated in the independent LIPGENE-Cordoba postprandial cohort of metabolic syndrome patients (n=75, mean age 56 y and BMI 33.4 kg/m2). In conclusion, we report for the first time that the common LEPR Gln223Arg genotype is an important predictor of postprandial TAG in males and our data highlights the importance of variables such as gender, when considering the population penetrance of common gene variants. The mechanistic basis of these associations remains to be established.

Molecular mechanisms underlying conjugated linoleic acid-induced skeletal muscle glucose transport

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Conjugated linoleic acid (CLA), a dietary lipid, has been proposed as an anti-diabetic/obesity agent. However, studies addressing the mechanisms of CLA on skeletal muscle glucose transport are limited. Our study investigated the cellular dynamics of cis-9, trans-11 (c9,t11) and trans-10, cis-12 (t10,c12) CLA isomers using L6 myotubes. Cells were treated without or with CLA isomers for 15 minutes and subsequently monitored for glucose uptake using isotope/fluorescently-labelled 2-deoxyglucose, intracellular Ca^{2+} (Ca_i^{2+}) release using Fluo-4 AM and GLUT4 translocation using immunofluorescence as well as protein phosphorylation events using Western blotting. Acute exposure of myotubes to CLA stimulated GLUT4 trafficking and glucose uptake by activating insulin-dependent signals, including phosphatidylinositol 3-kinase (PI3-kinase) p85 subunit and Akt substrate-160 kDa (AS160). Intriguingly, t10,c12-CLA stimulated Ca_i^{2+} release and phosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and AMP-activated protein kinase (AMPK) in a concentration-dependent manner, whereas c9,t11-CLA showed modest or no effects. Blocking PI3-kinase, Ca_i^{2+} release, CaMKII and AMPK abrogated CLA isomer-mediated AS160 phosphorylation and glucose uptake. Genetic knock down of CaMKII in myotubes using siRNA completely abolished CLA isomer-mediated glucose uptake. Furthermore, the evidence for a positive correlation between CaMKII and AMPK, in conjunction with inhibition of t10,c12-CLA-mediated AMPK activation by CaMKII blockers, indicates that CaMKII acts

upstream of AMPK. These data establish that t10,c12-CLA acts via Cai2+-CaMKII-AMPK-AS160 to stimulate skeletal muscle glucose transport, whereas the mechanism of c9,t11-CLA remains unclear.

Blood omega-3 concentrations are associated with reading, working memory and behaviour in healthy children aged 6-10 Years

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Background: Low blood omega-3 concentrations have often been reported in children with behaviour and learning disorders such as ADHD and dyslexia, but little is currently known about the possible links between omega-3 status and either behaviour or cognition in healthy children from the general population.

Objective: To investigate blood concentrations of omega-3 in relation to reading, working memory and behaviour in a healthy child population.

Procedure: 493 children aged 6-10 years from the DHA Oxford Learning and Behaviour (DOLAB) Trial gave a fingerstick blood sample for fatty acid analysis (Martek Biosciences Inc.), and were also assessed using age-standardised measures of reading, working memory and behaviour (ADHD-type symptoms as rated by parents and teachers). Correlations were performed between these measures and blood concentrations of omega-3 and omega-6 fatty acids, controlling for age, sex and socioeconomic status.

Results: Lower blood omega-3 concentrations were associated with poorer reading (DPA, $p<0.04$, DHA $p<0.02$, total omega-3 $p<0.02$) and poorer auditory working memory (EPA $p<0.004$, DPA $p<0.04$, DHA $p<0.002$, total omega-3 $p<0.003$). Lower DHA in particular was also associated with significantly higher scores for many behavioural problems as rated by parents (including oppositionality, hyperactivity-impulsivity, emotional lability and psychosomatic symptoms, all $p<0.01$), but only with higher anxiety on ratings by teachers ($p<0.05$). By contrast, omega-6 concentrations showed very few significant associations with either cognitive or behavioural measures.

Conclusion: Lower blood concentrations of omega-3 LC-PUFA (particularly DHA), predict poorer reading and auditory working memory performance, and more parent-rated behaviour problems, in otherwise healthy school children. These associations merit further investigation, ideally via well powered intervention studies that could establish whether improving omega-3 status might lead to benefits for child behaviour and learning in the general population.

Measurement of Resolvins and Protectins Using Liquid Chromatography-Tandem Mass Spectrometry: Comparison of Plasma and Serum Levels in Humans

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Background: The resolvins and protectins are a family of lipid mediators derived from the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). They are anti-inflammatory and have been implicated as endogenous mediators of resolution of inflammation.

Aim: To develop a method that simultaneously measures a number of resolvins and protectins using liquid chromatography-tandem mass spectrometry (LC-MS-MS) and to compare the levels of these metabolites in human plasma collected under different conditions and in serum.

Methods: Twenty healthy volunteers took 4g/day omega-3 fatty acids for 3 weeks and blood samples were collected into EDTA, citrate or heparin for the isolation of plasma, or prepared as serum. Samples were purified by solid phase extraction with LTB4-d4 as internal standard and analysed using LC-MS-MS on a Thermo TSQ Quantum Ultra system.

Results: The major EPA and DHA metabolites were ($\pm$)18-HEPE (m/z 317.2) and 17(S)-HDHA (m/z 343.1), respectively. Other DHA metabolites included 17(S)–RvD1; 17(S)–RvD2; 17(R)–RvD1; 10(R),17(S)-dHDHA and 10(S),17(S)-dHDHA. The assay limit of quantitation was 50pg/ml with linearity to 1000pg/ml. Intra- and inter-assay variability were 5% and 10% (18-HEPE) and 11% and 17% (17(S)-HDHA), respectively. Blood 18-HEPE levels were 430±43pg/ml (EDTA), 314±25pg/ml (heparin),

261±20pg/ml (citrate) and 257±24pg/ml (serum); and 17(S)-HDHA were 245±39pg/ml (EDTA), 127±21pg/ml (heparin), 156±32pg/ml (citrate) and 131±21pg/ml (serum).

Conclusions: Using LC-MS-MS permits simultaneous measurement of a number of resolvins and protectins in a single assay with excellent recovery and reproducibility. Our data suggest blood should be collected into EDTA for optimal measurement. This assay will allow determination of important mechanisms related to resolution of inflammation in atherosclerosis and other diseases.

Supplementation of n-3 fatty acid improved the symptoms of dry eye syndrome

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N-3 polyunsaturated fatty acid accumulates in high concentrations in the nervous system. Docosahexaenoic acid (DHA, 22:6n-3) found in the brain and retina in particular, plays an important role in their function. The purpose of this study is to determine the change of tear volume as the predominant symptom of dry eye syndrome in the dietary n-3 fatty acid deficient mice when compared to the n-3 fatty acid adequate mice. ICR mice were fed either an n-3 fatty acid deficient (n-3 Def) or adequate (n-3 Adq) diet for two generations. At 44 weeks of age in the second generation, the tear production of the mice was measured for 30 sec by phenol red-impregnated cotton threads. The tear volume in the n-3 Def mice was observed to be significantly lower (about 40% decline) than that of the n-3 Adq mice. In addition, the concentration of n-3 fatty acid in both the lacrimal and meibomian glands, which affects the production of tears, was markedly decreased compared to the n-3 Adq mice. However, the tear volume in the n-3 Def mice improved almost completely after one week of continuous administration of fish oil containing eicosapentaenoic acid (EPA, 20:5n-3) and DHA. These results indicate that dietary n-3 fatty acid deficiency increases the risk of dry eye syndrome, and suggests that n-3 fatty acid also has an important role in the production of tears.

Contribution of dietary intake and altered handling to alterations in omega-6 and omega-3 fatty acids in cystic fibrosis

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Individuals with cystic fibrosis (CF) have altered plasma fatty acids, which include lower linoleic acid (LA) and docosahexaenoic acid (DHA), with unaltered or higher arachidonic acid (ARA), and higher dihomo gamma linolenic acid (DGLA) compared to healthy individuals. The imbalance in n-6 and n-3 fatty acids has been implicated in the progression of the disease. Although the etiology of the altered fatty acids in CF is not fully understood, factors such as CF genotype, oxidative stress, and altered n-6 and n-3 fatty acid metabolism have been implicated. However, plasma n-6 and n-3 fatty acids are reflective of the dietary fat composition. Management of children with CF includes increased energy intakes, 120-150% of the usual recommendation, including higher fat intakes. The extent to which altered dietary fat content and composition contributes to altered plasma fatty acids in CF is not known. We determined the sources of energy, quantity, and quality of dietary fat and plasma phosphatidylcholine (PC) fatty acids for CF children (n= 74; aged 9.71 ± 0.39 yrs) and healthy children (n=73; aged 9.71 ± 0.44 yrs). Univariate analysis adjusting for weight and age showed total energy intake was higher (p<0.001), with 43.0± 6.9% and 35.0 ± 6.5 % from fat in CF and control children respectively, and a 1.3 folds higher dietary fat/protein ratio in CF than the control children (p<0.001). Fatty acid intakes also differed, with significantly higher saturated fatty acids and lower percent fat from eicosapentaenoic acid (EPA), LA , and DHA in CF children. Plasma PC showed higher EPA, but lower LA and DHA in the CF children (p=0.001). The differences in n-3 fatty acid intake may contribute to lower LA and DHA, but do not explain the retention of EPA in blood lipids in CF. (Supported by the Cystic Fibrosis Foundation)

Maternal fatty acid profile in relation to infant fat mass during the 1st year of life - results from the INFAT-study

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There is some evidence to suggest that the n-6/n-3 long-chain polyunsaturated fatty acid (LCPUFA) ratio in maternal nutrition may affect adipose tissue growth in the offspring.

We investigated the effect of n-3 LCPUFA supplementation and a concomitant reduction of the n-6 LCPUFA arachidonic acid (AA) intake in the diet of pregnant women/breastfeeding mothers on maternal/neonatal LCPUFA status and its relationship to infant fat mass up to 1 y of age.

In an open-label, randomized, controlled trial, 208 healthy pregnant women either received a dietary intervention [supplementation with 1200 mg n-3 LCPUFAs per day and a dietary counseling to reduce AA intake] from 15th week of gestation until 4 months of lactation or followed their habitual diet. Fatty acid profile was determined in plasma phospholipids (PL) and red blood cells (RBC) during pregnancy, lactation, and in umbilical cord blood. Multiple regression models adjusting for relevant confounders were performed to determine the relationship between maternal fatty acid composition and infant fat mass assessed by skinfold thickness measurements (SFT) and abdominal sonography up to 1 y pp.

Dietary intervention significantly reduced the n-6/n-3 LCPUFA ratio in maternal and cord blood plasma PL and RBC. Maternal RBC DHA, EPA and AA were significantly positively related to BMI at birth. RBC EPA was significantly positively related to BMI and body weight up to 1 y of age, too. No significant association of maternal LCPUFA status and fat mass assessed by SFT and ultrasonography was found during the 1st y of life.

Our results suggest that maternal DHA, AA and EPA serve as prenatal growth factors, while EPA also seems to stimulate postnatal growth. A reduced maternal n-6/n-3 fatty acid ratio does not appear to play a role in adipogenesis during the fetal and early postnatal period up to 1 y of age.

Cord blood omega-3 LCPUFA concentration increase with advancing gestation independent of maternal omega-3 supplementation in late pregnancy

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Background: Studies of cord blood fatty acid composition have consistently reported a high degree of heterogeneity, even in women consuming similar diets, however the contribution of non-nutritional determinants to cord blood lipid composition remains poorly defined. The aim of this study was to investigate the contribution of gestational age, parity, infant sex and maternal smoking to cord blood fatty acid concentrations at delivery in a large study population.

Methods: 2399 women were randmomized to receive capsules containing either DHA-rich fish oil (~800mg DHA/day) or vegetable oil (control) from 20 weeks gestation until delivery. Cord blood samples were collected from 1571 women (DHA, n=798, Control, n=773) at delivery, and levels of n-3 long chain polyunsaturated fatty acids (LCPUFA) Eicosapentaenoic acid (EPA), DPA and DHA, and n-6 PUFA Linoleic Acid (LA) and Arachidonic acid (AA) in plasma phospholipids determined. Linear regression models were used to investigate effects of non-nutritional determinants on cord blood fatty acid levels.

Results: In both the DHA and Control group there was a positive linear relationship between gestational age at delivery and the concentrations of DPA, DHA and total n-3 LCPUFA in cord blood phospholipids (P<0.0001). Conversely, the concentrations of LA and total n-6 PUFA decreased with increasing gestation (P<0.001). There was no effect of gestational age on the proportion of EPA or AA in cord blood

phospholipids. EPA and DHA levels were higher, and AA lower, in women in their first pregnancy compared to women with a parity ≥1. DHA levels, but not the levels of any other fatty acids, were higher in male compared to female infants (P=0.04).

Conclusions: This is the first study in a large study population to demonstrate the marked effect of gestational age at delivery on cord blood fatty acid composition, independent of maternal n-3 PUFA supplementation in late pregnancy. These findings have important implications for understanding the fatty acid requirements of pre-term infants.

Evidence of inadequate DHA during prenatal development contributes to loss of developmental potential in Canadian infants

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Docosahexaenoic acid (22:6n-3, DHA) is a long chain omega(n)-3 fatty acid, present in high concentrations in neural membranes. Experimental studies have shown that decreased brain DHA leads to histological, biochemical and functional deficits in neural function. Decreased brain DHA may result from inadequate n-3 or excessive n-6 fatty acid intakes. This study used a randomized, double-blind intervention with 400mg/day DHA or placebo (n=221) from 16 weeks gestation until infant delivery, to assess whether inadequate DHA during prenatal development limits neurologic development in infants. Infant development was assessed at 9, 14, and 18 months-of-age using tests of infant language, problem-solving, cognition and motor skill development. Risk of failure to achieve a test score in the upper quartile was assessed for infants in the placebo group, adjusting for confounding variables. Infant gender and maternal fatty acids were related to infant language development, with girls scoring higher than boys. As per the design, infants in the placebo group were less likely to achieve language development test scores in the highest quartile than infants in the DHA group at 14 and 18 months. The mean language, cognition, and motor development test scores between the placebo and DHA intervention group were not different. Mothers of infants with language scores in the highest quartile also had significantly higher erythrocyte phosphatidylethanolamine DHA or lower n-6 DTA+DPA/DHA ratios at 36 weeks gestation than all other infants. No differences between the placebo and DHA group, and no association with maternal DHA status were found for infant performance on the problem-solving task. The results of this study suggest that language development appears sensitive to the prenatal DHA supply, with tests with continuous outcome variables giving greater sensitivity to detect effects than pass/fail outcomes. Additionally, inadequate DHA supplies during gestation may limit potential infant development in our population. (Supported by CIHR).

LCPUFA status and body composition in 5 year olds and 19-20 year olds living in the Republic of Seychelles

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Background: The Seychelles Child Development Study (SCDS) is investigating the potential adverse effects on child neurodevelopment of prenatal methylmercury exposure from high maternal fish consumption during pregnancy. However, fish is also a rich source of long-chain polyunsaturated fatty acids (LCPUFA) and studies have shown that elevated plasma n-3 LCPUFA status is associated with a lower body mass index (BMI) in adults and children and a smaller waist circumference (WC) in adults.

Objective: To investigate associations between LCPUFA status and anthropometric measures in 5 year old children and 19-20 year old adolescents enrolled on the SCDS.

Procedure: Height and weight were measured and BMI was calculated in both age groups. Plasma phospholipid LCPUFA concentrations were measured using GC-MS. In the 19-20 year olds, WC and percentage body fat were also measured. Associations between n-3 LCPUFA (docosahexaenoic + eicosapentaenoic acid) status and these anthropometric measures were assessed using sex-specific

regression models controlling for socioeconomic status and birthweight. Models were repeated with the addition of total n-6 PUFA (linoleic + arachidonic acid) concentrations.

Results: Status of n-3 PUFA, with or without controlling for n-6 PUFA, did not predict anthropometric measures in either cohort. In both age groups, birth weight was a significant positive predictor of body weight in both sexes. Additionally, in both sexes birth weight was a significant positive predictor of BMI in the younger age group and WC, but not percentage body fat, in the older age group.

Conclusion: We did not find associations between n-3 LCPUFA status and anthropometric measures in this population of high fish consumers, which is not consistent with previous reports of beneficial associations of n-3 LCPUFA from fish and/or fish oils with fat mass in healthy adults. Rather we found, as others have done, that birth weight was a positive predictor of body weight and BMI.

Enzymatic activity and genetic variation in SCD1 modulate the relationship between fatty acids and inflammation

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Fatty acids (FA) represent a diverse class of molecules known to regulate inflammatory pathways. Therefore enzymes that regulate FA metabolism are attractive candidates to better understand the relationship between FA and inflammation. Stearoyl-CoA desaturase 1 (SCD1) is rate limiting for the conversion of saturated FA (SFA) to monounsaturated FA (MUFA). Evidence suggests that SCD1 activity may be positively associated with inflammation. Moreover, genetic variation in SCD1 may alter enzyme activity; however, it is unknown whether this affects inflammatory status. The goal of this study was to examine the relationships between plasma FA, SCD1 activity, and SCD1 polymorphisms with C-reactive protein (CRP) levels in young adults. SFA, MUFA, and CRP were measured in fasted plasma samples from European (n=279, 198 female and 81 male) and Asian (n=249, 179 female and 70 male) subjects, 20-29 years old. Circulating levels of palmitic (16:0), palmitoleic (16:1), stearic (18:0), and oleic acids (18:1) were measured by gas chromatography and SCD1 activity was estimated by the ratio of product to precursor (16:1/16:0; 18:1/18:0). Positive associations were identified between CRP levels and 16:0 (p < 2.0×10^{-4}), 16:1 (p<0.05), and the SCD1 index (18:1/18:0; p<6.0×10^{-3}) in European and Asian females, while 18:0 was inversely associated with CRP (p<2.0×10^{-4}) in both groups. Ten single nucleotide polymorphisms (SNPs) in SCD1 were genotyped in all subjects. One SNP (rs2060792) was associated (p<0.05) with 16:0 and 18:0 levels in females of European descent. This same SNP was also associated with CRP levels in both groups of females (p<0.05). Overall, SCD1 activity and genetic variation have an important role in modulating the relationship between FA and inflammation in young adults.

Eicosapentaenoic acid prevents cytokine production induced by palmitate via modulating long-chain acyl-CoA synthetase-1 expression in human THP-1 macrophages

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BACKGROUND AND OBJECTIVE Chronic inflammation caused by macrophages may be associated with progression of arteriosclerosis or obesity, both risk factors for cardiovascular events. In the Japan EPA Lipid Intervention Study (JELIS), Eicosapentaenoic acid (EPA), a n-3 polyunsaturated fatty acid, was found to reduce the incidence of cardiovascular events.

METHODS The effect of EPA on the expression of inflammatory factors induced by palmitate, a saturated fatty acid, was investigated using human THP-1 macrophages.

RESULTS Palmitate induced expression of inflammatory cytokines (TNFα and IL-1β) and activated nuclear factor-κB system similar to lipopolysaccharide (LPS). EPA strongly suppressed palmitate-induced up-regulation of inflammatory factors while slightly suppressing LPS-induced factors. Besides such inflammatory factors, palmitate and LPS both up-regulated expression of long-chain acyl-CoA synthetase 1 (ACSL-1), the enzyme that converts from palmitate to palmitoyl-CoA. While acyl-CoA synthetase

inhibitor and siRNA for ACSL-1 suppressed palmitate-induced TNFα expression, this inhibitor had no effect on LPS-induced TNFα expression. Thus, palmitate may stimulate cytokine production by mechanism different from that of LPS, which mediated through Toll-Like Receptor 4, and ACSL-1 may play an important role in this mechanism. Palmitate induced expression of SREBP-1 and ACSL-1, while EPA suppressed the expression of these genes.

CONCLUSION The suppressive effects of EPA on palmitate-induced cytokine production may be mediated by the suppression of ACSL-1 expression, at least partly. This anti-inflammatory effect of EPA may contribute to suppression of chronic inflammation caused by macrophages in atherosclerotic plaques.

Conjugated linoleic acid stimulates the production of functional HDL by intestinal cells

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By reverse transport, excess cholesterol moves from peripheral tissues effluxes to high density lipoproteins (HDL). This cholesterol is then taken up by means of SR-BI and excreted. The intestine is not only involved in the basolateral uptake of HDL-cholesterol and its apical secretion, it is also important for the biogenesis of HDL. We investigated the role of dietary fatty acids on intestinal reverse cholesterol transport and, more specifically, on HDL functionality. We used Caco-2 monolayers grown on transwells, which can synthesize HDL and can uptake HDL-cholesterol by means of SR-BI from the basolateral membrane. Cells were apically administered different isomers of conjugated linoleic acid (CLA) in mixed micelles and treated with BMS 212122 to inhibit chylomicron secretion. Cells were let to secrete HDL in the basolateral compartment for 24h in the absence or presence of an antibody to SR-BI (aSR-BI) which inhibits the interaction with HDL. After 24 hours, free and total cholesterol in the basolateral compartment were measured by GC-MS. The amount free cholesterol in the basolateral chamber in the presence of SR-BI was similar for all three isomers of CLA, but in the abscence of SR-BI cholesterol accumulated significantly more after treatment with c9,t11-CLA, suggesting a lack of reuptake of free cholesterol from the HDL previously secreted into the basolateral compartment, and the same was true for total cholesterol: 30% of free cholesterol and 57% of total cholesterol were retaken up by means of SR-BI when treated with t9,t11-CLA or 10,c12-CLA and only 13% of free cholesterol and 46% of total cholesterol were taken up after treatment with c9,t11-CLA. In summary, t9,t11- and t10,c12-CLA stimulate the production of HDL that are more efficient (aka functional) in delivering cholesterol to cells.

The Effect of Omega-3 Polyunsaturated Fatty Acids on Oral and Epidermal Squamous Cell Carcinoma

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Background: Squamous cell carcinomas (SCCs) of the aerodigestive tract often recur because of incomplete excision or the appearance of second primary or second field cancers. Recent evidence suggests that the omega-3 polyunsaturated fatty acids (PUFA) have antitumorigenic activities.

Objective: The potential of omega-3 PUFA to act as selective chemopreventive and therapeutic agents against oral and epidermal SCCs was tested and the mechanism of action was investigated.

Procedure and Results: The effect of omega-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on oral and epidermal malignant SCC, pre-malignant and normal keratinocytes was examined. The PUFA inhibited growth dose-dependently after 4 days, as measured by MTT cell viability assay. The PUFA selectively inhibited malignant and premalignant keratinocytes when compared to their normal and immortal counterparts. It was demonstrated that PUFA caused apoptosis by the annexin V apoptosis assay and cleavage of caspase 3 by western blotting. The cleavage of caspase 9 and 8 demonstrated the involvement of the intrinsic and extrinsic apoptotic pathways, respectively. Moreover, DHA and EPA decreased cell proliferation by the 3H-thymidine uptake assay. PUFA appeared to increase ROS production and DNA damage after 16 hours as well as JNK phosphorylation, especially at the higher concentrations. However, the use of anti-oxidants could not rescue the cells. PUFA caused a

rapid and sustained phosphorylation of ERK1/2 which was inhibited by MEK and EGF receptor inhibitors and was accompanied by an increase in COX-2 expression. No effect on Akt phosphorylation was observed. It is hypothesised that PUFA may cause the suprastimulation of EGFR and over-activation of ERK1/2 pathway which leads to apoptosis.

Conclusion: DHA and EPA display a marked anti-tumour effect against SCC keratinocytes at concentrations that do not eliminate normal cells, thus giving them a significant potential as future therapeutic and prophylactic tools against head and neck cancer.

Fish oil plus psychoeducation versus psychoeducation alone for attenuating posttraumatic stress symptoms among rescue workers after the Great East Japan Earthquake: A randomized controlled trial

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Background: On March 11, 2011, the Great East Japan Earthquake left about 20,000 dead or missing. Previous studies showed rescue workers are at high risk for posttraumatic distress, but no appropriate preventive strategy has been developed. This study aimed to determine whether fish oil supplementation could attenuate posttraumatic distress among Disaster Medical Assistance Team (DMAT) members deployed during the acute disaster phase following the Great East Japan earthquake.

Methods: In this single-blind, randomised, parallel-group trial, DMAT members who provided consent to participate were randomly allocated to a fish oil plus psychoeducation group or a psychoeducation alone group. Psychoeducation was provided to all participants in the form of a leaflet. The primary outcome was symptoms of posttraumatic stress disorder (PTSD) assessed by the Impact of Event Scale-Revised (IES-R) at 12 weeks after fish oil supplements were shipped to the participants. All analyses were by intention to treat.

Results: Of the 172 participants enrolled between April 2 and 12, 2011, 86 were assigned to each of the two groups. Only 1 participant in the psychoeducation alone group was lost to follow-up. When adjusted for age, sex, and IES-R score at baseline, no significant difference in primary outcome was seen between the two groups (-0.9, 95% CI, -3.0 to 1.2; P=.39). Remarkably, change in the IES-R score of women in the two groups from baseline to 12weeks was -3.9 (95% CI, -7.5 to -0.3; P=.04) when adjusted for age and IES-R scores at baseline.

Discussion: This trial did not show the effectiveness of fish oil supplementation for the prevention of posttraumatic stress symptoms in rescue workers. However, supplementation reduced PTSD symptoms significantly in women. Given the large number of survivors and limited number of psychiatrists available following large-scale disasters, fish oil supplementation may offer a safe strategy for preventing PTSD in women.

Investigating cellular membrane changes in human brain tissue during ageing using shotgun tandem mass spectrometry and 2D gel electrophoresis

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The degeneration of long-lived macromolecules within the human body has been proposed as the ultimate constraint on maximum human lifespan. Studies on long-lived biological structures such as the human lens have shown that there are significant alterations to both membrane lipid composition and the association of proteins with the cellular membrane as a result of age. The question is whether similar changes occurs in other long-lived, post mitotic cells present in the human body such as neurons. Currently there is limited knowledge available on the changes to the human brain lipidome with age, with most of our available data being obtained using older methods that are less sensitive and comprehensive than those now available. The aim of this project is to examine age-related changes to the lipid composition of cell membranes from human brain tissue using sensitive shotgun tandem mass spectrometry techniques. In addition, changes to the association of proteins with cellular membranes with age will be assessed through use of 2D gel electrophoresis. To our knowledge this will be the first concurrent application of these techniques on human brain tissue. Preliminary data from method

development using sheep brain tissue suggests that these two methods can be used on the same brain tissue samples with a high level of sensitivity. The use of newer, more sensitive mass spectrometry methods in the study of changes to membrane lipid composition during ageing will further our understanding of human brain lipidome, and may help in understanding of the mechanisms behind neurodegenerative disease associated with ageing such as Alzheimer's disease.

Meat from suckling lamb: a useful dietary source of essential fatty acids

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Lamb meat is the first meat recommended by Italian pediatricians in the weaning diet of children because is presumed to have a lower allergenicity compared with other red meat. Lamb meat at weaning showed positive effects in children with atopic dermatitis and multiple food hypersensitivity with a significant clinical improvement of the eczematous lesions. The use of lamb meat at weaning showed positive effects in the treatment of short bowel syndrome and Sandifer syndrome. The dietary lipids during the first years of life is important for optimal growth and for lifelong health. Of interest are arachidonic (ARA) and docosahexaenoic (DHA) acids for the development and function of the nervous and visual system. They derived from the diet or from conversion of their dietary precursors α-linolenic acid (ALA), and linoleic acid (LA). In this study, we compared the FA profile of fresh meat (FM) from Sarda suckling lambs (30 days of age) with those of commercial baby foods (BF) prepared with lamb meat. The results evidenced that the proportion of LA was higher in BF than FM probably related to the presence of vegetable oil (usually sunflower)l in some BF. The proportion of PUFA n-3 was higher in FM than BF, due to higher contents of ALA (3.55 vs 1.24), EPA (0.65 vs 0.10) and DHA (0.64 vs 0.04 mg/100 mg of FA). The ARA content was more than 6-fold higher in FM than BF (2.02 vs 0.32 mg/100 mg of FA). The n6/n3 ratio was markedly higher in BF than FM (14.3 vs 2.57). Results from this study suggest that meat from suckling lamb could be an interesting food in infant nutrition because can provide essential FA and long chain FA important for optimal neonatal development.

Analysis of omega-3 fatty acid content of South African fish-oil supplements

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Introduction: Substantial evidence describes the protective effects of marine derived omega-3 (n-3) polyunsaturated fatty acids (PUFA) on cardiovascular diseases as well as many other conditions. Numerous fatty acid preparations are marketed for supplementing the western diet which is low in long-chain n-3 PUFA. Since these preparations may vary in their long-chain n-3 PUFA content, we tested 46 commercially available products on the South African market for their fatty acid composition.

Method: Fourty five (45) commercially available n-3 fatty acid supplements were analyzed using gas liquid chromatography to determine their fatty acid content.

Results: More than half of the n-3 supplements available on the South African market contained less than 89% of the claimed eicosapentanoic acid (EPA) and/or docosahexanoic acid (DHA) content as claimed on the labels of the products. In order to meet the ISSFAL recommendation of 500 mg EPA+DHA per day can cost the South African consumer between R2 and R5 per person per day, representing an amount of R60-R150 per person per month. With regards to rancidity, the majority of capsules contained conjugated diene (CD) levels higher than vegetable oil obtained from opened containers (3 months) used for domestic cooking purposes, despite the addition of vitamin E as antioxidant.

Conclusion: Since no formal regulatory structure for dietary supplements currently exist in South Africa consumers must depend on self-regulation within the nutraceutical industry for assurance of product quality, consistency, potency and purity. Our results indicate that more than half of the n-3 fatty acid supplements on the South African market do not contain the claimed EPA and/or DHA contents as stated on product labels and contained CD levels higher than unused vegetable oils obtained from opened containers used for domestic cooking purposes.

Key words: Supplements, Eicosapentanoic acid (EPA), docosahexanoic acid (DHA), conjugated dienes (CD), fish oil.

Washout kinetics of eicosapentaenoic and docosahexaenoic acid from human plasma after supplementation with salmon oil

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Background: Recent research reported that eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) demonstrate diverse effects in the human body implicating different metabolic rates of EPA and DHA.

Method: Eight (n=8) randomly selected normolipidaemic subjects were loaded with fish oil capsules supplying 1 000 mg EPA+DHA/day over a 6 week period. After 6 weeks participants discontinued taking the fish oil capsules and were followed up for another 6 weeks. EPA and DHA were measured in plasma cholesteryl esters (CE), plasma triacylglycerols (TAG) and total plasma phospholipids (TPL). Dietary intake remained unchanged during the study period.

Results: The disappearance rates of EPA from TAG, CE and TPL all differed with TAG (half life = 3 days) demonstrating the fastest disappearance rate with TPL (half life = 5½ days) the slowest. In the TAG and TPL components, DHA levels were almost 2 to 3 times higher throughout the loading as well as washout period, when compared to the EPA levels. DHA disappeared from all plasma components at a slower rate than EPA and remained elevated for a longer period compared to EPA. No significant differences were observed in measurements for lipid oxidation such as conjugated diene (CD) and thiobarbituric acid reactive substances (TBARS) levels with (loading phase) or without (washout phase) omega-3 fatty acid supplementation.

Conclusion: Disappearance rates of EPA and DHA from plasma TAG, CE and TPL implicate different metabolic rates of these components. These finding may have important implications for human health especially in individuals with special dietary needs such as diabetes, cardiovascular disease and HIV.

Chronic and Acute Increases in Unesterified DHA are Protective in a Mouse Model of Neuroinflammation

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Background: DHA and its derivatives have anti-inflammatory, pro-resolving effects in several non-neural tissues. DHA reduces brain inflammation during ischemia and systemic inflammation, however, this may result indirectly from DHA attenuating the primary insult. The effect of DHA and its derivatives on neuroinflammation are not known.

Objective: To test the effects of increased brain DHA levels in a mouse model of neuroinflammation

Experimental Procedure: One acute infusion model and three chronic transgenic and/or feeding models were used to modulate the levels of brain DHA. To test neuroinflammation, at 12 weeks of age mice received an intracerebroventricular (icv) injection of lipopolysaccharide (LPS; 5 μg). Neuroinflammation was assessed 24 hours post-LPS by gene expression and immunohistochemistry. All fatty acids and neuroinflammatory markers were measured in the hippocampus.

Results: Using a transgenic model, we found higher levels of DHA in the phospholipid and unesterified pools of fat-1 transgenic mice compared to their wildtype littermates. Relative to wildtype littermates, fat-1 mice experienced an attenuated neuroinflammatory response and less neuronal degeneration. Feeding the wildtype littermates n-3 PUFA raised DHA levels in the phospholipid and unesterified pools to the same levels as fat-1 mice, and also resulted in a similar neuroinflammatory response. In a third chronic model, we achieved higher phospholipid but not unesterified DHA levels and there was no difference in neuroinflammation, highlighting the potential importance of the unesterified DHA pool. To isolate the

unesterified pool, we acutely administered unesterified DHA icv during the 24 hours post-LPS and found that it attenuates neuroinflammation. A similar attenuation was found when administering the 15-lipoxygenase product of DHA, 17(S)-hydroperoxy-DHA. Acutely infusing other fatty acids including arachidonic acid and docosapentaenoic acid n-6 did not decrease neuroinflammation, indicating the effect is unique to DHA.

Conclusion: Unesterified DHA attenuates hippocampal inflammation.

Effect of oxidized fish oil on oxidative stress markers and plasma lipidome profile. A randomized controlled trial in healthy subjects

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Intake of fish oil reduces the risk of coronary heart disease (CHD), and in CHD prevention healthy subjects are recommended two servings of fatty fish per week. n-3 supplements are recommended for those who do not include fish in their diet.Long chain n-3 fatty acids are susceptible to oxidation, and high content of oxidation products (PV >10 meq/kg and/or AV >20) have been reported in n-3 supplements. Whether intake of oxidized lipids leads to oxidative stress and unfavorable health effects is uncertain. Human studies investigating the health effects of intake of oxidized fish oil are lacking. In a double-blinded randomized controlled trial, 54 healthy subjects were assigned into one of three groups receiving capsules containing either 8 g/d of fish oil (1.6 g/d EPA+DHA), 8 g/d of oxidized fish oil (1.6 g/d EPA+DHA) or 8 g/d of high oleic sunflower oil (control). Fasting blood and morning spot urine samples were collected at week 0, 3 and 7. During the first three weeks of intervention, the subjects conducted a fully controlled isocaloric diet. Intake of fish and foods with n-3 fatty acids were not allowed in this study.We found no significant changes in markers of oxidative stress, lipid oxidation or inflammation (urinary 8-iso-prostaglandin F2α, plasma levels of 4-hydroxy-2 hexenal (4-HHE), 4-hydroxy-2-nonenal (4-HNE), and α-tocopherol, serum hsCRP or activity of antioxidant enzymes in erythrocytes) between the groups after three or seven weeks of intervention. However, plasma EPA and DHA increased significantly in both fish oil groups. The effect of fish oil with different quality was further investigated using a lipidomics approach. Preliminary results show altered lipid profile in the fish oil groups compared to the control group. It remains to be analyzed whether the lipid profiles differ between the fish oil groups, however these data will also be presented.

Palmitate induces phenotype changes in monocytes via de novo ceramide synthesis

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Ageing is associated with physiological changes such as altered adipose tissue distribution and redox status, elevated metabolic disorder risk and increased free fatty acids. Saturated fatty acids can induce insulin resistance in endothelial cells and monocytes, in part explaining the increase cardiovascular disease risk with elevated fatty acids. The aim of this study is to investigate the effects of the two major physiological fatty acids palmitate and oleate (saturated and monounsaturated fatty acids respectively) on monocyte phenotype. THP-1 monocyte cell surface marker expression, mitochondrial reactive oxygen species, cell viability and caspase-3 were evaluated following 24h treatment with palmitate, oleate or bovine serum albumin. A concentration dependent increase in CD11b ($p<0.01$), CD36 ($p<0.001$) and mitochondrial reactive oxygen species ($p<0.05$) following palmitate but not oleate treatment was determined by flow cytometry. Decreased metabolic viability ($p<0.01$) was observed with palmitate (300μM), whilst no significant change in caspase-3 was observed. The superoxide dismutase mimetic

MnTBAP (200μM) ameliorated the reduced metabolic viability and increased mitochondrial ROS due to palmitate, whilst increased CD11b and CD36 were unaffected. De novo ceramide synthesis inhibitor fumonisin B1 (50μM) prevented the palmitate dependent increase in CD11b (p<0.05) and CD36 (p<0.001). Ceramide converting inhibitors 1S,2R-D-erythro-2-N-myristoylamino)-1-phenyl-1-propanol (d-erythro MAPP 20 μM) and 1-phenyl-2-palmitoylamino-3-morpholino-1- propanol (PPMP 0.05 μM) were used to determine if ceramides or downstream sphingolipids are required for palmitate induced phenotype changes in monocytes. In the presence of MAPP and PPMP, palmitate induced increase in CD11b are sustained suggesting sphingolipids are key players in the observed phenotypic changes. The present study demonstrates in monocytes that palmitate but not oleate increases cell surface marker expression, accompanied by increased mitochondrial ROS and decreased metabolic viability. MnTBap could ameliorate changes in mitochondrial ROS and metabolic viability, but surface antigen expression decreased only by fumonisin B1 suggesting changes require de novo ceramide synthesis

The Role of Nuclear Factor kB on the Synthesis of Lipid Inflammatory Mediators on THP-1 Derived Macrophages

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Background: The inflammatory signaling cascade activated by LPS has been widely described previously. Nuclear factor κB (NFκB) is a transcription factor that works synergistically with other elements to determine the production of pro-inflammatory mediators by macrophages. The cytokines and newly characterized lipid metabolites synthesized by macrophages may determine the extent and duration of an inflammatory response. Nevertheless, the synergy between these elements remains to be elucidated.

Objective: To determine the coordination between NFκB activation and the kinetics of cytokine and lipid metabolite synthesis following LPS stimulation.

Procedure: THP-1 cells were differentiated to macrophages using PMA. Macrophage phenotype was confirmed by surface protein expression. THP-1 derived macrophages were incubated with different concentrations of LPS for different times. Cell pellets and supernatants were collected and NFκB activation, and cytokines and lipid metabolites evaluated.

Results: PMA treatment induced the expression of macrophage features on THP-1 cells. LPS incubation resulted in a dose- and time-dependent increment of inflammatory cytokine production which was positively related to NFκB pathway activation, as evidenced by decrease of IκB-α expression on the cytosol and increment of nuclear p65 expression at early time points.

Conclusion: Our findings suggest that in THP-1 macrophages, the activation of NFκB pathway is responsible for cytokine production and may orchestrate the synthesis of lipid metabolites that determine the course of the inflammatory response.

Stearidonic Acid (SDA) from soybeans affects the EPA levels in red blood cells

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Stearidonic acid (SDA) enriched soybean is a land-based, sustainable source of omega-3 fatty acids, which could help meet the recommendations of many professional organizations for increased intake of long-chain omega-3 fatty acids. SDA is a product of delta-6 desaturase, the rate limiting enzyme in the conversion of alpha-linolenic acid (ALA) to eicosapentaenoic acid (EPA). Through biotechnology, soybeans have been enriched with SDA omega-3 fatty acid. To provide support for SDA from soybean as an alternative source of dietary long-chain omega-3 fatty acid, we conducted a series of studies. We first demonstrated that SDA increases the percent of EPA in red blood cells (RBC %EPA) in volunteers using encapsulated SDA enriched soybean oil or SDA ethyl esters (Lemke et al., Am. J. Clin. Nutr., 2010). Next, a dose and time course study indicated that a consumption of encapsulated SDA ethyl esters increases the RBC %EPA, which followed a first order kinetic model (Krul et al., Prostaglandins

Leukot Essent Fatty Acids., 2011 Epub). Recently, SDA enriched soybean oil was used as an ingredient in everyday foods and consumed by healthy men and women (21-65 years old). In this 12-week study, we assessed the impact of SDA in foods on the RBC %EPA to demonstrate whether SDA soybean oil is an alternative omega-3 fatty acid source. Volunteers consumed an average of 8.0 g/day of oil in two baked bars and one dairy beverage. Subjects in the SDA group had significantly higher RBC %EPA than the control group. Consistent with encapsulated SDA studies, SDA in food increased RBC %EPA with 22% efficiency of EPA alone. Furthermore SDA is metabolized to EPA three to five times more efficiently than ALA in humans. Results of these studies combine to support SDA oil as an alternative source of long-chain omega-3 that can be formulated into everyday foods.

Effect of omega-3 fatty acid supplementation in patients with rheumatoid arthritis: a 16-week, double-blind, placebo controlled study

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N-3 polyunsaturated fatty acids (PUFA) have anti-inflammatory effects and may be useful for the treatment of inflammatory diseases such as rheumatoid arthritis. We examined the efficacy of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) supplements on rheumatoid arthritis on the top of standard anti-inflammatory treatment. Patients with rheumatoid arthritis were randomized into two groups in a double-blind, placebo-controlled parallel designed multicenter study. Patients received the respective 5 capsules of either n-3 PUFA (2.090 g of EPA and 1.165 g of DHA) or sunflower oil with high oleic acid for 16 weeks. Clinical status and dietary intake were evaluated and blood samples were taken at week 0 and 16. Differences before and after intervention were tested with paired t-test. One hundred seven patients were randomized, 80 finished, and 14 each with n-3 PUFA and placebo dropped. No side effect and dietary intake of fish or n-3 PUFA were found. Omega-3 Index (sum of EPA and DHA in erythrocytes) was significantly ($p<0.001$) increased in n-3 PUFA group (from 9.03 +/- 0.35 % to 11.61 +/- 0.38%), but not in placebo group (from 8.49 +/- 0.29% to 8.52 +/- 0.29%). Dose of nonselective nonsteroidal anti-inflammatory drugs (NSAIDS) was significantly ($p=0.039$) decreased in n-3 PUFA group. However, patient's and physician's global assessment, pain scale, morning stiffness, and Korean Health Assessment Questionnaire were not significantly changed in patients with n-3 PUFA and placebo. Our study suggested that incorporation of EPA and DHA in membrane could be an attractive treatment for rheumatoid arthritis by reducing NSAIDs consumption.

Estimated intake of dietary oxidized lipids based on NHANES 2003-2006: Higher intakes are associated with lower markers of adiposity

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Previous studies in animals have shown that consumption of high amounts of highly oxidized fats promote weight loss and reduce adipose mass but increase glucose intolerance. No one knows how much of these oxidized lipids are consumed by humans and if they influence adipose mass and glucose homeostasis. In this study we wanted to estimate the consumption of dietary oxidized lipids (DOL) in the US by using the NHANES 2003-2006 data and to determine the relationship DOL consumption and markers of adiposity. The NHANES food frequency questionnaire was used to identify food items that are likely to contain DOL and the average amount of DOL in each of these foods was determined based on previously published data. Intake of DOL was categorized into "low" or "high" based on median intake. Our results demonstrate that a greater number of women (55%) compared to men (45%) consume higher amounts of oxidized lipids. In a similar fashion a higher DOL intake is seen among younger subjects (2-18y) compared to older subjects (>18y); among Hispanics and non-Hispanic blacks compared to non-Hispanic whites; and, among subjects with a lower poverty income ratio compared to that of a higher PIR. Surprisingly, those in the "high" consumption group had significantly lower levels of markers of adiposity such as body fat, triceps skinfolds and waist circumference. However, mean plasma insulin and glucose levels were not different between the "low" and "high" consumers. Finally, multivariate analysis demonstrated that high DOL intake is significantly and inversely associated with BMI, body fat, triceps and waist circumference. Furthermore, higher level of DOL consumption was associated with

hyperglycemia (β=1.11, p≤0.001). This is the first study to suggest that among slightly over-weight subjects higher intakes of DOL are associated with lower markers of adiposity but with higher levels of plasma glucose.

Assessment of the HS-Omega-3 Index and breast milk DHA using dried samples collected on filter paper

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Assessment of the omega-3 fatty acid status of both human blood and breast milk is important in studies that examine the relationships between these fatty acid levels and health outcomes. Both blood and milk samples are difficult to collect and process in the field, hence improved approaches to sample acquisition are needed to expand the research base for these metrics. We have therefore developed methods to collect, preserve, transport and analyze the fatty acid composition of dried blood spots (DBS) and dried milk spots (DMS). For both sample types, a single drop of blood/milk were applied to Whatman 903 cards which had been pretreated with an antioxidant preservative cocktail (OxyStop®). Frozen human milk samples (n=5) were thawed and analyzed immediately or spotted on cards and placed in a drawer at room temperature for 5 days. The DHA content of the liquid vs. dried milk samples were analyzed in triplicate. Mean DHA levels were 0.19% and 0.20%, respectively, with an r2 = 0.99 (p<0.01). RBC EPA+DHA (the HS-Omega-3 Index®) was estimated from DBSs vs. direct RBC analysis in 106 healthy subjects. The correlation was r2 = 0.92 (p<0.0001). Thus both RBC and milk omega-3 content can be accurately assessed from samples collected and transported on filter paper.

The effects of aliquot size and time at -20°C on erythrocyte fatty acid content

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Background: Red blood cell (RBC) fatty acid (FA) patterns have been shown to predict risk for cardiovascular and other chronic diseases. As part of a project analyzing >8,000 RBC samples from the Women's Health Initiative (WHI) Memory Study we observed in some samples implausibly low levels of highly unsaturated fatty acids (HUFA) suggestive of degradation. This was hypothesized to be due to short term storage at -20°C during aliquot preparation at the central processing laboratory.

Objective: To examine the effects of RBC storage at -20°C on FA composition.

Methods: One-hundred WHI samples, that had always been stored at -80°C, were subjected to similar conditions (amount of time at -20°C and storage in 80uL and 250uL aliquots) as the affected samples. Additional experiments incorporating antioxidant treatment were conducted.

Results: Sample degradation occurred at -20°C with the average HUFA loss in the 80uL (-5.9% per wk) being greater than that in the 250uL aliquots (-3.5% per wk). Significant losses occurred even in samples moved from -80°C to -20°C. The saturated and monounsaturated FA increased in a compensatory manner. Storage with antioxidants largely prevented the HUFA loss.

Conclusions: RBC FA composition is adversely affected by even short term storage at -20°C but is stable at -80°C. Larger aliquot sizes are more resistant to oxidation than smaller.

Comparative Effects of ALA, EPA, and DHA in Diet-Induced Obese Rats

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The three major dietary n-3 PUFA, α-linolenic acid (ALA; C18:3n-3), eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3), may produce distinctly different responses on the risk factors for metabolic syndrome. However, the paucity of comparative studies involving all three individual n-3 PUFA provides a weak basis for assuming different responses in the pathophysiology of chronic diseases. In this study, we have shown that ALA and EPA/DHA produced different physiological responses to decrease the risk factors of the metabolic syndrome in high-carbohydrate, high-fat diet-induced obese rats. At the same dosage, ALA did not reduce total body fat but induced lipid redistribution

away from the abdominal area and decreased glucose tolerance, insulin sensitivity, dyslipidemia, hypertension, and left ventricular dimensions, contractility, volumes and stiffness. EPA and DHA increased sympathetic activation, reduced the abdominal adiposity and total body fat and attenuated insulin sensitivity, dyslipidemia, hypertension and left ventricular stiffness but not glucose tolerance. However, ALA, EPA and DHA all reduced inflammation in both the heart and the liver, cardiac fibrosis and hepatic steatosis. Since the physiological responses to EPA and DHA were similar, it is likely that the effects are mediated by DHA with EPA serving as a precursor. Also, ALA supplementation increased DHA concentrations but induced different physiological responses to EPA and DHA. This result strongly suggests that ALA has independent effects in the metabolic syndrome, not relying on its metabolism to DHA.

Metabolic and clinical effects of low n-6 and low n-6 plus high n-3 dietary interventions in Chronic Daily Headache patients

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Background: Targeted dietary interventions are a promising strategy for alleviating physical pain. We tested whether altering dietary n-6 and n-3 PUFAs in chronic headache patients could alter the abundance of lipid mediators linked to nociception, as well as their fatty acid precursors.

Methods: 68 patients with chronic headaches were randomized to one of two dietary interventions for 12 consecutive weeks. Group 1 reduced dietary n-6 LA and AA and consumed average US amounts of n-3 fatty acids. Group 2 reduced dietary LA and also increased ALA, EPA and DHA intake. Our diet method integrated the following elements: (1) provision of foods accounting for two-thirds of calories; (2) diet counseling; (3) self-monitoring; and (4) an intervention-specific website. Extensive laboratory and clinical data were collected at baseline and every 4 weeks.

Results: 56 of 68 randomized participants completed the intervention. Fatty Acids: Group 1 (n=28) reduced erythrocyte LA (-16.2%, $p<0.01$) and %n-6 in HUFA (-4.3%, $p<0.01$), and increased EPA (+32.3%, $p<0.01$), DHA (+15.6%, $p<0.01$) and the n-3 Index (+12.8%, $p<0.01$). Group 2 (n=28) reduced LA (-14.3%, $p<0.01$), AA (-15.1%, $p<0.01$) and %n-6 in HUFA (-22.8%, $p<0.01$), and increased EPA (+273%, $p<0.01$), DHA (+89%, $p<0.01$) and the n-3 index (+97%, $p<0.01$). Lipid Mediators: Group 1 had a reduction in 11-HETE (-18%, $p=0.05$) and a trend toward reduction in 8-HETE (-16%, $p=0.06$). Group 2 had reductions in 5-HETE (-30%, $p<0.01$), 8-HETE (-25%, $p<0.01$), 11-HETE (-29%, $p<0.01$) and 12-HETE (-19%, $p=0.02$).

Conclusion: Dietary n-6 lowering independently altered erythrocyte fatty acid composition and reduced certain AA metabolites. The addition of dietary ALA, EPA and DHA to a low n-6 intervention produced more marked alterations in fatty acid composition and AA metabolites. Analyses of clinical endpoints (e.g. headache frequency, intensity and quality-of-life), and other metabolic mediators are pending and will be available for presentation.

Structural Characterization of Saturated Branched Chain Fatty Acid Methyl Esters by Collisional Dissociation of Molecular Ions Generated by Electron Ionization

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Branched chain fatty acids (BCFA) are saturated fatty acids (FA) present in a wide range of biological samples, such as bacterial membranes, human skin, and secretions. BCFA are also produced by rumen bacteria and thus are components of rumen tissue and milk FA. The traditional approach for identifying branching in BCFA methyl esters (BCFAME) is to examine the electron ionization (EI) mass spectrum (MS). However, this method does not always enable a confident assignment of the methyl branch. Zirrolli and Murphy (1993) showed that the analysis of molecular ions of a limited number of BCFAME by tandem MS yield characteristic fragments upon collisional dissociation using a triple quadrupole instrument. Our aim was to extend their results by analysis of 30 BCFAME from vernix caseosa, lanolin,

and pure multimethyl BCFA, using a tabletop 3-D ion trap for activated molecular ion EI-MS/MS (EI-MS/MS).

iso-BCFAME, which have a methyl branch on the n-2 carbon, produce prominent ions corresponding to the loss of the terminal isopropyl group in the molecule [M-43] (M-C3H7). Anteiso-BCFAME, which have a methyl branch on the n-3 carbon, produce prominent ions corresponding to the loss of the terminal ethyl [M-29] (M-C2H5) and the terminal isobutyl [M-57] (M-C4H9) groups. The characteristic fragments described above for iso-BCFAME and anteiso-BCFAME are found in short chain BCFAME as well as in long chain BCFAME. Furthermore, anteiso-BCFAME, but not iso-BCFAME, produce more prominent m/z 115 peaks corresponding to a cyclization product around the ester. As with anteiso-BCFAME, dimethyl and polymethyl BCFAME yield fragments corresponding to losses on both sides of methyl branches that are more than 6 carbons away from the FA carboxyl end.

In conclusion, we show here a convenient and reliable method for assignment of branching in methyl BCFAME. Using EIMS/MS produces characteristic ions that enable confident structural assignment of BCFAME.

Branched Chain Fatty Acid Content of United States Retail Cow's Milk and Implications for Dietary Intake

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Branched chain fatty acids (BCFA) are primarily saturated fatty acids (FA) with one or more methyl branches on the carbon chain. In recent studies we showed that BCFA are present in the gut of normal human newborns, reduce the incidence of intestinal disease, and affect gut microbiota in rat pups. BCFA are native to the human gut and they are bioactive nutritional agents, thus their presence in the US food chain is of enhanced interest. Ruminant food products are expected to be the major contributor of BCFA in the American food supply. We report the distribution of BCFA in US retail milk and estimate BCFA intake in the American diet based on intake of dairy and beef. Conventionally produced whole milk samples were obtained from 56 processing plants across the US. Milk fat was processed by standard methods and analyzed using a gas chromatograph (GC) and a GC coupled to a mass spectrometer (GC-MS). BCFA comprise 2%, w/w of milk fat FA, and 3% of milk fat saturated FA. Milk BCFA have chain lengths of 14–18 carbons, of which 58% is composed of anteiso-BCFA. From these results and from USDA food availability data, we estimated that the daily per capita BCFA intake of Americans is about 220 mg/d from dairy, greater then the average daily 100 mg/d consumption of docosahexaenoic acid and eicosapentaenoic acid. If current dietary recommendations were followed, this estimate would rise to 400 mg/d, and would comprise 0.6% of the recommended total fat intake in a 2000kcal diet. Adding intake from beef consumption, these estimates rise to 400 and 575 mg/d, respectively. We conclude that BCFA intake exceeds that of several bioactive FA and may be highly variable, depending on dairy and beef intake.

The DHA Oxford Learning and Behaviour (DOLAB) Study: A randomised controlled trial of DHA supplementation in healthy children

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Evidence from small clinical trials has indicated that an increased dietary intake of omega-3 LC-PUFA may have benefits for various aspects of child behaviour and learning. To date, almost all such trials have focused on clinical groups with specific developmental disorders such as ADHD, dyslexia, DCD or autism. What has not yet been addressed is whether any benefits of omega-3 supplementation may extend to the general healthy school population.

We therefore designed a randomized, double-blind, placebo-controlled trial involving healthy children aged 6 – 10 years (n=360) recruited from mainstream state schools in Oxfordshire, UK. Inclusion criteria were: underperforming in literacy skills (<20th centile on standardized reading test) but other abilities

within the normal range. Exclusion criteria were: major learning disabilities or medical disorders, medications known to affect behavior or learning (e.g. ritalin), taking omega-3 supplements or eating fish ≥ 2 x week, English not the first language.

Interventions were as follows: Active treatment: Fixed dose of 600 mg DHA (from algal oil, Martek Biosciences Inc), delivered in 3 x 500 mg capsules/day, each providing 200 mg DHA. Placebo: 3 x 500mg capsules/day containing high-oleic sunflower oil, matched with active treatment for taste and colour. Duration of treatment was 16 weeks, with delivery of capsules via schools during term-time and parents at other times.

Primary outcomes were: Reading performance and Working Memory (Recall of Digits) from the British Ability Scales and Behaviour (ADHD-type symptoms) assessed via Conners' Parent and Teacher Rating Scales.

This study has recently been completed (n=362) and results from primary outcomes will be presented.

Dietary fatty acid intake and factors of cardiovascular risk in adolescence and young adulthood

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Background: Primary prevention of cardiovascular diseases and metabolic disorders should begin at early age. Information on frequency of early cardiovascular risk factors in the young is rare.

Objective: With the aim to evaluate associations between cardiovascular risk and dietary and lifestyle factors and of identifying types of effective prevention strategies, adolescents and young adults were included in a population- based study in the city of Leipzig-Lipid Study Leipzig (LSL).

Procedure: The study included measurement of cardiovascular risk factors, i.e. parameters of lipid metabolism, anthropometric parameters, blood pressure, and the evaluation of dietary and lifestyle factors of 725 adolescents between 14 and 18 years and 1221 young adults (age group 19 – 30 years). Evaluated seven day diet diaries were used to assess the composition of daily dietary intake and analysed with the computer program PRODI 4.5 expert (adolescents n = 252, young adults n = 303).

Results: Even in adolescence and young adulthood, there were significant associations between cardiovascular risk factors, such as overweight, high blood pressure and atherogenic lipoproteins. Overweight was significantly related to elevated non-HDL-cholesterol and higher blood pressure, indicating already the disposition for glucose intolerance. The mean total intake of fatty acids was largely in accordance with general recommendations, although the composition could be improved. The composition of dietary fatty acids was 46% saturated fatty acids, 38% monounsaturated fatty acids, and only 16% polyunsaturated fatty acids. Especially the mean intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of adolescents was low (0.16 g / d).

Conclusion: The dietary intake of polyunsaturated fatty acids, especially of omega-3 fatty acids is inadequate. The public health challenge is achieving adoption of beneficial fatty acid supply in the setting of influences that promote unhealthy lifestyles in adolescence and young adulthood.

Dietary PUFA Reductions Decrease Non-enzymatic Oxidative Stress Biomarkers in Humans

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Background: Polyunsaturated fatty acids (PUFAs) are potential targets for free-radical mediated, non-enzymatic oxidation. Alterations in dietary PUFAs may modulate oxidative stress in vivo by modifying the oxidative susceptibility of lipid substrate pools. We therefore investigated whether lowering dietary PUFAs reduces biomarkers of non-enzymatic oxidative stress in humans.

Methods: After a 4-week baseline phase, 68 patients with chronic headaches were randomized to consume one of two low PUFA dietary interventions. Group 1 reduced dietary n-6 PUFAs and consumed average US amounts of n-3 PUFAs. Group 2 reduced dietary LA and also increased ALA, EPA and DHA intake. Our diet method integrated the following elements: (1) provision of foods accounting for two-thirds of calories; (2) diet counseling; (3) self-monitoring; and (4) an intervention-specific website. Three

biomarkers of free-radical mediated oxidative stress (9-HETE, F2-isoprostane, and malondialdehyde) were measured in plasma at baseline and at the conclusion of the 12-week intervention. 9-HETE was measured by LC/MS/MS; F2-isoprostane and malondialdehyde were measured with ELISA.

Results and Discussion: 56 of 68 randomized participants completed the intervention (n=28 per group). Participants in both groups had significant reductions in plasma 9-HETE (Group 1, -24%, p=0.008; Group 2, -36%, p<0.001). Results for F2-isoprostane and malondialdehyde are pending and will be available for presentation. Low PUFA diets reduced 9-HETE, a biomarker of non-enzymatic oxidative stress, in human plasma. The 9-HETE precursor arachidonic acid (AA) was unchanged in Group 1 (data not shown), indicating that the observed reductions in 9-HETE were not due solely to a reduction in precursor abundance. These findings and pending results for F2-isoprostane (AA-derived) and malondialdehyde (not specific to AA) provide insights into mechanisms linking dietary PUFAs and oxidative stress in humans.

n-3 PUFAs increase cholera toxin induced lipid raft size in vitro and ex vivo

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n-3 Polyunsaturated fatty acids (PUFAs) remodel plasma membrane organization; however, mechanistic details remain unclear. Here we studied the effects of n-3 PUFAs on cholera-toxin induced lipid raft formation in cell culture and in animal models using quantitative imaging (TIRF, FRET) and biochemical methods. Treatment of EL4 cells with docosahexaenoic acid (DHA), but not eicosapentaenoic acid (EPA), increased lipid raft size and modified protein lateral organization. Biochemically, DHA incorporated directly into specific phospholipids of rafts and displaced cholesterol from raft-like to non-raft membranes. We then tested our in vitro model at the animal level by feeding mice fish oil (FO) enriched diets, modeling human intake, for 3 weeks. Relative to controls, the FO diet also increased lipid raft size of B cells. However, FO had no effect on cholesterol lateral distribution and did not heavily incorporate into raft-like membranes. Instead, FO increased surface levels of gangliosides and increased plasma membrane order relative to no cross-linking. Finally, the enhancement in membrane order with FO was accompanied by an increase in B cell protein clustering on the nanometer scale. Taken together, our data show that in vitro and in vivo incorporation of n-3 PUFAs increased the size of lipid rafts to manipulate protein lateral organization; however, there were some differences between the model systems at the biochemical level.

Inhibitors of FATP-mediated fatty acid uptake have different activites in cells that are tissue-specific models for fat, liver, muscle, intestine and pancreas

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Metabolic syndrome, insulin resistant diabetes, and cardiovascular disease are currently major contributors to the burden of heath management in developed countries. The etiology of these diseases is still poorly understood. A growing body of evidence suggests that dysregulation of fatty acid metabolic pathways is a major causative factor. Currently, only a limited number of drugs are available to combat these diseases and it is clear new drugs, which more narrowly target the metabolic pathways involved, are required. In the present work, we characterized two compounds called CB5 and CB16 previously identified in a high throughput screen of 100,000 compounds that inhibit fatty acid uptake mediated by HuFATP2. Kinetics of inhibition for these two were measured in five cell lines that are models for intestinal epithelia (Caco2), hepatocytes (HepG2), muscle (C2C12), pancreatic ß-cells (INS-1E) and adipocytes (3T3-L1 and primary human adipocytes). The measured IC50s were quite variable between cell lines, with the highest sensitivity being for INS-1E (pancreatic ß-cells) with IC50 of 82nM for CB5 and 4uM for CB16. The sensitivity to drugs was correlated with expression lelvels of FATP2 in each line. The lowest sensitivity was for adipocytes with IC50 of 900 uM and 58 uM for CB5 in 3T3-L1 cells and human adipocytes, respectively. The IC50s for CB16 were 68uM and 36uM in the same cells. The compounds did not cause toxicity or affect other cellular activities such as glucose uptake when applied up to 1mM. Thus, CB5 and CB16 are good candidates as mechanistic inhibitors of fatty acid uptake and may be useful to develop drugs to prevent lipotoxicity.

Fatty acid desaturase indices, metabolic syndrome components and insulin resistance in children

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Background: Fatty acid composition, which affects chronic inflammation relating to the development of metabolic syndrome (MetS), is influenced not only by the dietary fat intake but also by desaturating enzymes: stearoyl-CoA desaturase (SCD), delta-6 desaturase (D6D) and delta-5 desaturase (D5D). We analyzed fatty acid composition in plasma phospholipids and obtained the desaturase indices to investigate their associations with the components of MetS and insulin resistance in Japanese children.

Methods: The study subjects were 237 children (115 boys and 122 girls) aged 11.5 ± 1.5 years (mean $\pm$ SD). The fatty acid composition of plasma phospholipids was analyzed and the desaturase indices were determined: SCD (16:1n-7/16:0: SCD16 and 18:1n-9/18:0: SCD18), D6D (20:3n-6/18:2n-6) and D5D (20:4n-6/20:3n-6).

Results: D6D and D5D indices, but not SCD16 or SCD18 indices, were significantly associated with triglyceride levels, high-density lipoprotein cholesterol levels, waist-to-height ratio and insulin resistance in both sexes. In addition, the clustering of MetS components showed a significant association with increased D6D and decreased D5D indices.

Conclusions: The n-6 polyunsaturated fatty acid desaturation pathway might contribute to the development of MetS. D6D and D5D indices could provide a new approach to establish personalized fatty acid nutrition.

The Role of Omega-3 Polyunsaturated Fatty-Acids in Programming Inflammation-Resolution

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Macrophages are important to study in the context of inflammation given that their physiological locality situates these cells to respond to the initial inflammatory insult. Dietary supplementation with omega-3 polyunsaturated fatty acids (n-3 PUFAs) have been shown to decrease levels of inflammatory mediators produced by macrophages, yet little is known with regard to their therapeutic mechanism of action(s). This study is set to determine if the introduction of n-3 PUFAs promote inflammation-resolution as a result of modifying the functional phenotype and/or molecular pathway profile of activated macrophages during an inflammatory cascade.

As a model for macrophage immune cell function, RAW264.7 cells, a murine-derived macrophage cell line, was exposed to inflammatory stimuli following pre-treatment with n-3 PUFAs. Cells were treated with different doses/combinations of alpha linolenic acid (ALA), decosahexaenoic acid (DHA), and eicosapentaeonic acid (EPA) prior to macrophage activation to assess the combinatory impact of dietary n-3-PUFAs. To model an inflammatory event, RAW 264.7 were stimulated with bacterial lipopolysaccharide (LPS). Cell culture supernatants and cell lysates were collected and analyzed for different biomarkers of inflammation. The tests performed included Griess reaction (to measure the production of nitrite) and western blots (to measure levels of inflammatory proteins).

A decrease in the inflammatory markers cyclooxygenase-2 and inducible nitric oxide synthase were detected by western blot analyses with various concentrations and combinations of ALA, DHA, and EPA, compared to control samples. The most significant decreases in nitrite levels were observed in cultures treated with EPA and DHA. Current studies are underway to evaluate changes in the functional phenotype of macrophages (i.e. phagocytic ability, TNF-alpha expression) following n-3 PUFA treatment and LPS activation.

Understanding the biological role of n-3 PUFAs in inflammation will advance the knowledge-base into the therapeutic role it can play in reducing inflammation and developing novel therapies to treat chronic inflammatory diseases.

North-South-Disparity in Omega-3-Index of Subjects from Different Cities of Germany

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Objectives: Nutrition surveys repeatedly show that fish consumption and consequent EPA+DHA intake in Germany is higher in northern coastal regions compared to other regions. In the following study we investigated differences in the omega-3-Index of subjects from four German cities.

Material and methods: Presented omega-3-Index data were the baseline levels from a population taking part in a recent six-month intervention trial, where the effect of different omega-3 fatty acid supplements on lipid levels of hyperlipidemic subjects (mean age: 61.3 years) was investigated. 150 subjects were recruited in Hamburg (n=40, north), Hannover (n=63, middle), Goslar (n=19, middle) and Munich (n=28, south). Subjects additionally completed a food frequency questionnaire (FFQ) to obtain information on dietary intake.

Results: The mean omega-3-Index level in the total study population was 7.23±1.70%. Additionally, we observed slight differences between cities, with highest levels in Hamburg (7.97±1.94%), followed by Goslar (7.01±1.53%), Hannover (6.99±1.51%) and Munich (6.88±1.62%). The differences were significant between Hamburg and Hannover (p=0.005) as well as between Hamburg and Munich (p=0.018). The omega-3-Index levels were not correlated with the estimated fish intake from FFQ.

Conclusions: We identified regional differences in the omega-3-Index of a German population with higher levels in Hamburg (coastal region) compared to Hannover and Munich (both distant from the coast). A reason for a lacking connection between the omega-3-Index and fish intake could be the insensitiveness of the used FFQ, which did not distinguish between lean and fatty fish. A further explanation is that fish intake is only one causative factor for the variability of the omega-3-Index. The omega-3-Index levels in the study population were slightly higher than expected and close to levels that are considered as optimal (>8%), possibly because the selected cardiovascular risk study population was characterized by a higher nutrition awareness and thus adequate dietary omega-3 fatty acid intake.

No Correlation Between Omega-3 Index Increase and Triglyceride Decrease in Hypertriglyceridemic Patients After Fish Oil Supplementation: Results From a Randomized Controlled Trial

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Long-chain n-3 FAs from fish oil (FO) are known to effectively lower plasma triglyceride (TG) levels and to increase the percentage of EPA and DHA in membranes of red blood cells (omega-3 index, O3I). However, whether there is a relationship between O3I increase and TG decrease had remained unclear.

This question was investigated using data from a previously performed double-blind, randomised placebo-controlled trial in 150 hypertriglyceridemic patients over three months: Study participants were allocated to one of three groups: 1) FO concentrate with EPA+DHA (1.0g + 0.67g daily) as re-esterified TG (rTG-group); or 2) corn oil (placebo-group); or 3) FO concentrate with EPA+DHA (1.0g + 0.67g daily) as ethyl-esters (EE-group). O3I and plasma TG were measured at baseline and after three month. No changes in O3I or TG levels were observed in the placebo-group. However, while O3I increased significantly in both n-3 FA-treated groups from baseline to three month (EJCN 2011, 65(2):247-54), TG levels significantly decreased from baseline to three month only in the rTG-group (PLEFA 2011, 85(6):381-6).

Further analysis revealed that there was no significant correlation between O3I increase and decrease in plasma TG in any group. This appeared to be due to high inter-individual variations and comparatively low mean TG levels. Nevertheless, using a model of linear regression without a constant, it can be verified that the TG reduction is significantly associated with the O3I decrease in both the rTG- and EE-group. Deviations from this model can be especially observed in participants with low TG baseline levels.

Increases in O3I and decreases in plasma TG levels, although occurring at the same time, and caused by the same intervention, were not found to correlate. We conclude that plasma TG levels are not a viable substitute for the O3I to monitor treatment with EPA+DHA.

The impact of membrane lipid composition on macrophage activation in the immune defense against Rhodococcus equi and Pseudomonas aeruginosa

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Objective: Nutritional fatty acids are known to have an impact on membrane lipid composition of body cells, including cells of the immune system, thus providing a link between dietary fatty acid uptake, inflammation and immunity. In this study we reveal the significance of macrophage membrane lipid composition on gene expression and cytokine synthesis thereby highlighting signal transduction processes, macrophage activation as well as macrophage defense mechanisms.

Procedure: RAW264.7 were supplemented with PUFA in a concentration of 15 µmol/l for 72h. In the last 24h of incubation cells were stimulated with LPS, PMA or with viable bacteria of the genera R. equi and P. aeruginosa. Gene expression analysis was performed by means of a SYBR Green-based quantitative RealTime PCR. Genes analyzed include the genes for the surface molecules Fc receptor, MHCII and CD86, the adapter proteins MyD88 and RICK as well as the antimicrobial peptide lysozyme. Cytokines were detected in cell supernatants using suitable ELISA kits.

Results: We identified PUFA of both the n-3 and the n-6 family to down-regulate the synthesis of (i) the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, (ii) the co-stimulatory molecule CD86 as well as (iii) the antimicrobial polypeptide lysozyme. At this, the action of the fatty acids partially depended on the activation status of the macrophages. It is particularly important to note that the immune-suppressive action of the PUFA could also be seen in case of infection of RAW264.7 with viable microorganisms of the genera R. equi and P. aeruginosa.

Conclusion: Our data raise the possibility of a directed supply of fatty acids to immunocompromised individuals as a supportive therapy of chronic infections caused by persistent pathogens.

Serum fatty acid synthase (FASN) from breast cancer patients and its association with nutritional status and fatty acid consumption

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INTRODUCTION Fatty acid synthase (FASN) is the key-enzyme for de novo fatty acid biosynthesis and it is serum concentration is known to be elevated in cancer patients. It is not clear if nutritional status or dietetic fatty acids are associated with FASN presence under this circunstance.

PURPOSE To evaluate breast cancer patients' fatty acid synthase (FASN) serum concentration and establish its relationship with nutritional status and fatty acid consumption.

METHODS Case control, cross-sectional study with 18 newly diagnosed breast cancer patients (BC) and 29 cancer free controls (CG). Nutritional status was assessed with BMI, waist circumference and percentage of body fat percentage. Mean food intake was obtained with two non-consecutive 24-hour recalls using 5-step multiple pass method (USDA). Plasma FASN antigen was measured by ELISA (FASN-detect™, Baltimore, USA). Statistical analyses were carried out by parametric, nonparametric and Spearman's correlation tests.

RESULTS Patient's mean age were 46.8 ± 9.7 years (BC) and 44.4 ± 8.6 years (CG), the mean BMI were 28.2 ± 4.9 kg/m2 (BC) and 29.4 ± 6.9 kg/m2 (CG). An increased FASN serum concentration was found in BC (132.51 ± 95.05 ng/dL) compared to controls (36.88 ± 20.87 ng/dL) (p <0.0001). Fat consumption was lower in BC, but there was no qualitative difference in fatty acid intake among groups, except for lower relative linoleic acid (LA) intake by BC compared to CG (p <0.03). We observed negative correlation between FASN and docosahexaenoic acid (DHA) intake by CG (ρ = - 0.503, p = 0.03). Plasma FASN did not associate with nutritional status.

CONCLUSION FASN concentration was increased among breast cancer patients but it didn't show correlation with fatty acid intake. Among healthy women, dietary DHA was negatively associated with FASN plasma concentration.

Mechanisms of the relationship between essential fatty acid blood status, cognitive performance and cardiovascular function in young adults

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In order to support a healthy lifestyle, a properly balanced diet should contain adequate levels of essential fatty acids (EFA's).   EFA's cannot be manufactured through bodily processes, and are obtained from dietary sources. Important EFA's include those of the omega 3 (N3) family, including EPA & DHA, and the omega 6 (N6) family, including AA. A balanced ratio of n-3 and n-6 is essential for the body to function efficiently, however typically modern Western ratios are around 16:1. While research suggests that EFA status is related to both cognitive performance and cardiovascular function, there is little research investigating possible associations between these variables.The current study investigated the mechanisms of the relationship between EFA status, cognitive performance and cardiovascular function. It was hypothesised that a healthier EFA status would correlate with improved cognitive performance and increased blood flow velocity, and that improved cognition would be mediated by increasing blood flow velocity. A group of 34 students participated in the study conducted at Swinburne University, Melbourne Australia. Methodology included a computerised cognitive test battery, transcranial Doppler ultrasound, SphygmoCor pulse wave analysis and plasma phospholipid analysis. EFA status was significantly correlated with aspects of both cognitive function and cardiovascular health. Specifically, two EFA variables were significantly correlated with blood flow velocity through the common carotid artery, and EFA status was significantly correlated with multiple cognitive variables. No cardiovascular measures significantly correlated with any cognitive measures. This data suggests that a healthy EFA status has a positive effect on cardiovascular function and cognitive performance. However, cardiovascular variables were not correlated with cognitive measures. This may suggest that improved cognitive function is not mediated through improved cardiovascular functioning, however more research is needed. Further research, with a larger, more representative sample and more sophisticated statistical analysis, is required to validate these results.

Neuroactive Steroids Measured by a Novel Mass Spectrometry Platform

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Neuroactive steroids have been implicated in a variety of disorders including epilepsy, multiple sclerosis, neurodegenerative diseases, traumatic brain injury, depression, schizophrenia, alcohol dependence, pain and anxiety disorders. Several neuroactive steroids are potent modulators of the GABAA receptor and consequently can alter the excitability of the central nervous system. Measurements of these analytes are most commonly approached by gas-chromatography mass spectrometry (GC-MS) methods requiring chemical derivatization to decrease polarity and increase sample volatility. Here we present a novel quantitative non-derivatizing LC-MS/MS assay to screen a panel of seven C-21 neuroactive steroids in plasma from women classified either as low risk (LR) or high risk (HR) to postpartum depression (PPD). The pilot-feasibility study measured plasma concentrations from six cohorts from each group and were measured at three gestational age time points and 3-9 weeks post-partum. The assay set out to measure plasma concentrations of progesterone, deoxycorticosterone, 5☐-dihydroprogesterone, 5☐-dihydroprogesterone, allopregnanolone, allotetrahydroxycorticosterone, pregnanolone, in addition to GABA (separate LC-MS/MS assay). The LOQ for progesterone was determined to be ~200 pg/mL, comparable to the ~100 pg/mL value commonly reported for GC-MS values. The corticosterones were not detected above the assay LOQ for any of the samples. However, preliminary data suggest that allopregnanolone, a potent modulator of the GABAA receptor, is elevated for the HR over the LR group; 61.2 versus 13.7 ng/mL for the <36 hours cohorts, respectively. For GABA, increasing GABA levels were positively correlated to gestational age and into the postpartum (p=0.0029). Further, HR subjects had an average GABA level 0.34µg/mL lower than LR subjects (p=0.78). Results for the pilot study will be

presented along with a discussion of measuring neuroactive steroids by alternative LC-MS/MS approaches.

Lipase catalyzed synthesis of DHA rich free fatty acids from Salmon Fish Oil

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World food market is currently interested in foods that provide not only nutritive values but also health benefits to humand. Therefore, the social demand is to focus on the synthesis of compounds which can prevent diseases such as dyslexia, dyspraxia, Alzheimer & Schizophrenia and heart diseases by simultaneously modulating inflammation, cytokine release, immune response, platelet aggregation, vascular reactivity, thrombosis, and allergic phenomenon. The present paper investigates on the hydrolysis of Salmon fish oil with immobilzed Candida antractica lipase-B (CAL-B) to produce free fatty acids. The study has been carried out at the optimized conditions such as pH 7, 1:1 (w/w) solvent to oil ratio as iso-octane, 3:1 (w/w) water to oil ratio, 35 deg. C temperature, 500 U (equivalent to 0.133g) of CAL-B. The ping-pong bi-bi mathematical model was investigated and found satisfactory for the hydrolysis of fish oil without inhibition by water. The study of activation energy (E) reflects that reaction occurs without mass tansfer limitations. CAL-B was found to give 45 % triglycerides conversion to free fatty acids after 3 runs at optimized conditons.

Key words: Free fatty acids, salmon fish oil, Candida antractic lipase-B, hydrolysis.

An analysis of plasma triglycerides and tissue levels of fatty acids, tocopherols, and sterols in guinea pigs after parenteral lipid emulsion infusion

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Lipid emulsions (LEs) are made by mixing vegetable or fish oils with egg yolk, and, therefore, contain different types and amounts of fatty acids (FAs), tocopherols, and sterols. In addition to providing fuel calories, different bioactive components of LEs are expected to modulate metabolism. We, therefore, analyzed plasma triglyceride (TG) and tissue (liver, heart, lung, RBCs, kidney and adipose) levels of FAs, tocopherols and sterols after intravenous infusion in guinea pigs, an animal model that closely resembles the human relative to its LDL/HDL constituents. Intralipid, ClinOleic, Omegaven, and SMOFlipid LEs were infused (5 ml) daily (over a one-hour period) into guinea pigs for 10-days and compared to animals receiving an oral-chow diet. All LEs were well tolerated and plasma ALP/ALT/AST activities did not increase following any of the LE treatments. The plasma TG levels did not increase over that of the control group for any of the LE-infused. Tissues exhibited FA profiles reflecting the LEs as early as 6 hours post-infusion. The tissues exhibited enrichment of 18:2(n-6),18;1(n-9), and 20:5(n-3)+22:6(n-3) after infusion of Intralipid, ClinOleic, and Omegaven, respectively. The plasma n-3-FA profile more closely mirrored the hepatic FA profile, whereas RBC lipid levels were qualitatively reflective but not quantitatively reflective of FA levels in other tissues. Alpha-tocopherol (alpha-T) was the major tocopherol isomer in plasma and the tissues. None of the LEs increased alpha-T levels over that of the control animals; however, Intralipid depleted alpha-T from liver and plasma, whereas Omegaven depleted alpha-T from plasma only. ClinOleic, being lowest in total tocopherol content, nevertheless preserved the alpha-T levels both in liver and plasma. ClinOleic increased squalene levels in liver, whereas no changes in plasma squalene levels were seen. ClinOleic, Intralipid, and Omegaven caused a moderate increase in hepatic cholesterol levels. None of the emulsions caused elevated cholesterol levels in the plasma.

Postoperative atrial fibrillation in relation to DHA and AA levels in plasma phospholipids and red blood cell membranes

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Background: Data from recent controlled trials using n-3 long-chain polyunsaturated fatty acids (LC-PUFA) to prevent postoperative atrial fibrillation (POAF) following open heart surgery suggest that high levels of these fatty acids in plasma phospholipids (PPL) and RBC membranes (RBCM) may be associated with an increased risk of POAF.

Objective: To examine the relationship between the levels of n-3 LC-PUFA and n-6 LC-PUFA arachidonic acid (AA) in PPL or RBCM and POAF.

Methods: We combined data from two recent randomized trials of similar design examining the effect of treatment with n-3 LC-PUFA in preventing POAF. Fatty acid levels were measured immediately prior to surgery. Multivariable logistic regression was used to examine the independent relationship between the levels of fatty acids in PPL or RBCM and POAF.

Results: A total of 362 patients were enrolled with median age 66.4 (range, 38.3-85.4) years, 76.5% were male, and 68.8% underwent CABG only. The overall incidence of POAF was 44.8%. The POAF group exhibited lower levels of AA in PPL and RBCM, and a higher level of docosahexaenoic acid (DHA) in RBCM than the sinus rhythm group ($P < 0.05$). After adjusting for confounding variables, POAF was associated with RBCM AA levels (($P = 0.05$, OR 0.88 (95% CI 0.76 - 1.00) for each percent of AA in RBCM). With the 4th quintile of DHA as a reference group, the lowest three quintiles and the highest quintile were significantly associated with increased risk of POAF, ($P = 0.04$, OR 1.87 (1.02-3.42) and $P = 0.01$, OR 2.64 (1.26-5.52), respectively).

Conclusion: Our study shows a novel association between POAF and AA levels that may possibly be explained by electrophysiological effects of this fatty acid. The association between DHA and POAF seems to be complex and appears to follow a U shaped relationship, suggesting that high DHA levels may confer a pro-arrhythmic effect.

Relationship of inflammatory mediators to n-6 and n-3 long-chain polyunsaturated fatty acids in plasma phospholipids and red cell membrane lipids following open heart surgery

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Background: A systemic inflammatory response occurs in patients undergoing cardiac surgery. The balance between pro- and anti-inflammatory mediators may partly depend on the status of n-6 and n-3 long-chain polyunsaturated fatty acids (LC-PUFA).

Objective: To examine the relationship of n-6 and n-3 LC-PUFA in plasma phospholipids (PL) and red blood cell (RBC) membrane lipids with inflammatory mediators before and following open heart surgery.

Methods: Blood samples from patients undergoing open heart surgery (n = 168) were collected at baseline, immediately prior surgery (preoperatively), and on the third postoperative day for fatty acid analysis and assessment of inflammatory mediators.

Results: At baseline, higher plasma PL level of eicosapentaenoic acid (EPA) was associated with lower concentration of high-sensitivity C-reactive protein (hs-CRP), and higher docosahexaenoic acid (DHA) level was associated with lower interleukin-12 (IL-12) and IL-18 concentrations. In RBC, higher arachidonic acid (AA) level was associated with higher concentration of tumor necrosis factor β (TNF-β), whereas higher DHA level was associated with lower IL-18 concentration. Plasma levels of interferon-γ (IFN-γ) and TNF-β were lower and those of IL-6, IL-8, IL-10, IL-18 and hs-CRP were higher on the third postoperative day than preoperatively. Higher preoperative level of AA in plasma PL was associated with greater increase in IL-10 and lesser increase in TGF-β, whereas higher level of EPA was associated with lesser increase in IL-10 following surgery. In RBC membrane, higher preoperative AA level was

associated with more pronounced decrease in TNF-β, and lesser increase in TGF-β, whereas higher level of EPA was associated with greater increase in IL-1 β and TGF-β.

Conclusion: Our findings support the notion that n-6 and/or n-3 LC-PUFA in plasma PL and/or cell membrane lipids play an important role in modulating the inflammatory response following surgery.

Effects of iron and n-3 fatty acid supplementation on spontaneous motor activity and ADHD-related behaviour in iron-deficient primary school children in South Africa

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Objectives: To investigate the effects of iron and n-3 fatty acid (n-3 FA) supplementation on spontaneous motor activity and ADHD-related behaviour in iron-deficient (ID) primary school children.

Design: A 2x2 factorial, randomized, double-blind, placebo-controlled trial in ID primary school children aged 6-10 years (n=321). Subjects were randomly assigned to receive one of the following supplement combinations: (1) Docosahexaenoic/eicosapentaenoic acid (DHA/EPA, 420mg/80 mg) + iron (50mg as ferrous sulphate); (2) DHA/EPA + placebo; (3) placebo + iron; (4) placebo + placebo. Supplements were provided four times per week for 8.5 months (excluding school holidays). Physical activity was recorded in a subsample (n=105) at baseline, midpoint and endpoint during three different time periods namely during morning class time (08h00–10h30), break time (10h30-11h00) and after-break class time (11h00-12h00). Classroom behaviour of study subjects was assessed by teachers' questionnaires at baseline and endpoint.

Results: There were no significant interactions of time point or time period with treatment. However, there was a significant main effect of DHA/EPA supplementation for lower morning class time activity at endpoint (P=0.024). Biological markers indicating better or poorer iron status were positively and negatively associated with activity at break time, respectively. Subjects in the group receiving both Fe and DHA/EPA supplements showed a significant improvement from baseline to endpoint on the cognitive problems/inattention subscale (P=0.002) of the Conners' Teacher Rating Scale (CTRS). At endpoint, morning class time activity was positively associated with all CTRS subscale scores (higher scores indicate worse behaviour), except for the cognitive problems subscale.

Conclusion: These findings suggest that n-3 FA supplementation may influence ADHD-related behaviour during class time. Furthermore, the accelerometer might be a useful tool for assessing both classroom and break time activity behaviour in school children.

Treatment with Docosahexaenoic Acid, but not Eicosapentaenoic Acid Improves Mitochondrdial Function in Genetic Cardiomyopathy

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Treatment with a mixture docosahexaenoic ccid (DHA) and eicosahexanoic Acid (EPA) is beneficial in heart failure patients, but the mechanism is not clear, nor is it known if both DHA and EPA are required. Cardiac mitochondrial dysfunction contributes to LV dysfunction and pathology in heart failure, as seen in greater susceptibility to mitochondrial permeability transition pore (MPTP) opening, which prevents ATP production and triggers cell death. Here we evaluated the hypothesis that DHA is superior to EPA in improving mitochondrial respiration and preventing Ca2+-induced MPTP opening in a gentic model of heart failure. Male cardiomyopathic hamsters (Bio TO-2 strain), 6 wks of age, were assigned to either standard chow, or either DHA or EPA at 0.7 and 2.1% of energy intake (equivalent to 1.5 and 4.5 g/d in humans) (n=12/group). At 30 wks mitochondria were isolated from subsarcolemmal (SSM) and interfibrillar (IFM) compartments, and respiration and Ca2+-induced MPTP opening were assessed. Respiration was lower in IFM in CM compared to the normal hamster, and they were more sensitive to MPTP opening (figure). There were no differences in SSM among groups. Dietary supplementation with DHA or EPA had no effect on survival, LV function (assessed by echo) or LV mass. Treatment with the high dose of DHA increased state 3 respiration in IFM with succinate as the substrate (P=0.03), and

delayed Ca2+-induced MPTP to values similar to normal hamsters (P<0.01). In a subsequent series we initiated treatment with high dose DHA (2.1% energy intake) in 30 wk old animals and observed improved mitochondrial respiration and left ventricular function. In conclusion, a high dose of DHA, but not EPA or a low dose of DHA, increased mitochondrial oxygen consumption and delayed MPTP opening. This suggest that clinical trials in heart failure patients should assess treatment with a high dose DHA without EPA.

The infusion of docosahexaenoic acid during reperfusion after ischemia has dose dependent effects in isolated perfused rat hearts

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The antiarrhythmic benefits of docosahexaenoic acid (22:6n-3, DHA) are documented but DHA may also reduce cardiac injury during post-ischemic recovery. Presently, different concentrations of DHA were infused during reperfusion immediately following no flow ischemia in isolated rat hearts. The effect on heart function and ischemic injury was examined and contrasted to the effects of similar concentrations of palmitic acid. Hearts were excised from chow-fed (AIN-93M), male, Spague Dawley rats (9-12 wks of age) and then perfused via a Langendorff preparation. Hearts were allowed to adjust for 30min followed by 30min no flow ischemia. Following ischemia, DHA or palmitate complexed with bovine serum albumin in buffer was infused for 15 min at 0, 10, 20, 40, 60, 80, 100 and 120 nmol/mL followed by an additional 75 min of reperfusion. Heart functional data was recorded continuously. Whole heart infarct volume was determined after staining with triphenyltetrazolium chloride. The infarct size after 10 nmol/mL DHA treatment was smaller than the infarct sizes after vehicle and 10nmol/mL palmitate control (P = 0.02 for both by LSD post hoc after significant F-value for 2-way ANOVA). The infarct sizes after 20, 40 and 60 nmol/mL for DHA and palmitate were similar to vehicle. With 80, 100 and 120 nmol/mL infusions, the infarct sizes tended to be increased above vehicle and the lower dose fatty acid infusions. Functional assessments indicate that contracture increased dramatically with the higher fatty acid infusion concentrations. Contractility was higher with low fatty acid infusions and lower with high fatty acid infusions, as compared with vehicle. Interestingly, several hearts became fibrillate and died during infusion with 120 nmol/mL of DHA specifically. The present findings indicate that some of the cardiac benefits of DHA may be provided during post-ischemia reperfusion, but these benefits are dose dependent.

Study of the role of GPR120 in pancreatic beta-cells.

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The cell surface free fatty acid (FFA) receptor, GPR120, has been proposed to mediate the cytoprotective actions of long-chain unsaturated fatty acids in the murine intestinal L-cell line, STC-1. Unsaturated FFAs also promote cytoprotection in pancreatic β-cells but it is not clear whether this response is mediated via GPR120. The present study has examined this possibility using the rodent pancreatic β-cell line, INS-1E.

RT-PCR analysis revealed that GPR120 is expressed at the mRNA level in INS-1E cells and in primary human islets. In both cases, the shorter of the two known isoforms was present. INS-1E cells were then manipulated by stable transfection to generate clones conditionally over-expressing or under-expressing GPR120, respectively. Changes in GPR120 expression did not affect the insulin-secretory response to glucose (25mM) or to depolarisation with 30mM KCl.

Unexpectedly, over-expression of GPR120 resulted in changes to the morphological phenotype of the cells, which displayed dramatically increased adhesion to the surface. Analysis of gene expression profiles revealed changes in several adhesion-related molecules under these conditions, including ICAM1.

Incubation of wild type INS-1E cells with palmitate (C16:0) resulted in a dose-dependent loss of viability (EC50~ 50µM) but no difference in either the potency or the magnitude of this response was found in cells having altered expression of GPR120. In agreement with earlier findings, the mono-unsaturated

FFA, palmitoleate (C16:1) and MUFA derivative oleoylethanolamide each protected β-cells against palmitate-induced toxicity in a dose-dependent manner. Again, neither the potency nor the extent of cytoprotection was affected significantly in cells with altered expression of GPR120.

The results suggest that changes in expression of GPR120 may lead to alterations in the expression of cell adhesion molecules in pancreatic β-cells. However, they imply that this receptor does not influence the ability of long-chain FFAs to regulate cell viability in these cells.

Role of Hepatic Monounsaturated Fatty Acid Synthesis in Metabolic Regulation

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Stearoyl-CoA desaturase (SCD) catalyzes the de novo synthesis of monounsaturated fatty acids (MUFA) from saturated fatty acid precursors. Past work demonstrated that SCD1 deficiency impairs hepatic lipogenesis and protects against diet-induced obesity. Our objective was to determine if hepatic MUFA synthesis is sufficient to restore the impaired lipogenic program in SCD1 global knockout mice (GKO). To address this, we produced liver-specific transgenic mice expressing either human SCD5, which preferentially synthesizes oleate (18:1n-9), or mouse SCD3, which preferentially synthesizes palmitoleate (16:1n-7), and introduced these transgenes into GKO mice. Hepatic oleate synthesis largely prevented very-low-fat diet-induced weight loss and increased white adipose tissue weight to a greater extent than hepatic palmitoleate synthesis. In females, hepatic SREBP-1 maturation and lipogenic gene expression increased in hSCD5/GKO while expression of these genes remained lower in mSCD3/GKO mice. Additionally, hepatic oleate increased plasma glucose levels more than hepatic palmitoleate synthesis did. Overall, this work suggests that hepatic MUFA are involved in the regulation of hepatic lipogenesis and gluconeogenesis with oleate being more potent than palmitoleate. Supported by NIH.

Exposure to a maternal n-3 fatty acid-deficient diet during brain development provokes excessive hypothalamic-pituitary-adrenal axis responses to stress and mental disorders in male rat offspring later in life

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Brain docosahexaenoic acid (DHA, 22:6n-3) accumulates rapidly during brain development and is essential for normal neurological function. The aim of this study was to evaluate whether DHA deficiency leads to dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis in response to stress and whether the period of brain development was the critical time at which this occurs later in life. Rats were exposed to an n-3 fatty acid-deficient or an n-3 fatty acid-adequate diet either throughout the pre-weaning period from embryo to weaning at 3 week-old or during the post-weaning period from 3- to 10-week-old. We found that exposure to the n-3 fatty acid-deficient diet during the pre-weaning period resulted, at weaning, in a significant decrease in hypothalamic DHA levels and a reduced male offspring body weight. DHA deficiency during the pre-weaning period significantly increased and prolonged restraint stress-induced colonic temperature changes and serum corticosterone levels, caused a significant increase in GABAA antagonist-induced heart rate changes and enhanced depression-like behavior in the forced-swimming test and anxiety-like behavior in the plus-maze test in later life. These effects were not seen in male rats fed the n-3 fatty acid-deficient diet during the post-weaning period. These results suggest that DHA deficiency leads to excessive HPA responses to stress and to mental disorders in adulthood and that the critical period for this to occur is pre-weaning. We propose that these effects of hypothalamic DHA deficiency during brain development may involve a GABAA receptor-mediated mechanism.

Mind-Body Interface: Polyunsaturated fatty acids and somatic symptoms in major depressive disorder

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Background. Lower n-3 polyunsaturated fatty acids (n-3 or omega-3 PUFAs) levels and genetic variations on their metabolic enzymes of PUFA metabolic enzymes, phospholipase A2 (PLA2) and cyclo-

oxygenase-2 (COX2), have been found to be associated with the risk of depression (1-4). In this study, we aimed to examine specific roles of n-3 PUFAs, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and the polymorphisms on PLA2 and COX2 in different clusters of depressive symptoms.

Methods. Patients with major depressive disorders (n=122) and their healthy controlled subjects (n=122) were assessed to examine the effects of PUFA levels and single nucleotide polymorphisms (SNPs) of PLA2 BanI and COX2 rs4648308 genes on the development of major depression and on specific clusters of depressive symptoms.

Results. Patients with major depressive disorders had a significant lower level of EPA (p=0.03) and a trend of lower level of DHA (p=0.08). The COX2 rs4648308 AG genotype was associated with a higher risk of major depression (p=0.006; odds ratio=2.36, 95% CI=1.27-4.40), while the PLA2 BanI GG genotype had a borderline effect (p=0.06; odds ratio=1.81, 95% CI=0.87-3.79). The "at risk" COX2 polymorphism was associated with more somatic symptoms (p=0.003) and lower DHA (p=0.002), and the "at risk" PLA2 polymorphism was associated with more somatic symptoms (p=0.025). In addition, lower EPA and DHA levels were both significantly correlated with more somatic symptoms in patients with depression.

Conclusions. Genetic variations in the COX2 and PLA2 genes have effects on depression and somatic features, possibly by affecting the levels of EPA and DHA. N-3 PUFAs may be a potential biomarker to understand clinical subtypes of depression (1).

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Abnormal rod and pigment epithelium cell activity in ELOVL4 transgenic mice are associated with decreased retina levels of docosahexaenoic acid and very long chain fatty acids (C24-C36)

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Autosomal dominant Stargardt macular dystrophy (STGD3), an early onset form of macular degeneration, is caused by a mutation in the ELOVL4 gene (ELongation Of Very Long chain fatty acids type 4). The ELOVL4 transgenic mouse model of STGD3 (Karan et al., 2005 102:4164-9) shares similar pathologic features with the human counterpart. However, a complete retina fatty acid profile has yet to be described in this STGD3 model. One-month-old wildtype (WT) and ELOVL4 transgenic mice were fed a nutritionally complete semi-purified diet for 5 months. Whole retina fatty acid composition was analyzed at 1 and 6 months by micro- and fast- gas chromatography. Fatty acid profile was correlated with retina anatomy and function as assessed with immunohistochemistry and electroretinogram (ERG) recordings, respectively. Compared with WT, 6 month old ELOVL4 mice had 13% (w/w) lower retina levels of docosahexaenoic acid (DHA, C22:6n-3) and 11.5% higher levels of arachidonic acid (AA, C20:4n-6). ELOVL4 mice also had lower n-3 hexaenoic C32 and C34 levels, contributing to an overall 34% lower level of total n-3 very long chain fatty acids (VLCFA, C24-C36). The n-3 VLCFA to LCFA ratio (an indicator of VLCFA synthesis from shorter precursors) was significantly decreased in the ELOVL4 mice. Signs of retina degeneration were only seen in 6 months ELOVL4 mice and consisted of specific rod and RPE dysfunction (reduced mixed scotopic rod a-wave and c-wave amplitude, respectively) and anatomical loss of rods (8 instead of 10 photoreceptor rows). These results indicate that a specific decrease in n-3 fat levels (mainly DHA and n-3 VLCFA), might contribute to the pathological changes affecting rods and RPE in the ELOVL4 mouse model of STGD3. Our results support further studies of DHA and VLCFA supplementation as a mean to prevent the progression of macular degeneration as occurs in STGD3.

Homozygous sickle cell patients supplemented with DHA and EPA have reduced urinary albumin

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Background: Sickle Cell Disease (SCD) is a systemic inherited blood disorder which causes chronic inflammatory ischemic-reperfusion injury to kidneys. Studies have suggested that supplementation with DHA protects against kidney injuries and improves renal function in patients with glomerulopathy.

Objectives: The objective was to investigate the effect of supplementation with the omega-3 fatty acids, DHA and EPA, on renal function of patients with homozygous Sickle cell disease.

Procedure: Steady-state homozygous sickle cell patients on continuous supplementation with DHA and EPA for two years (n=34) and non-supplemented controls (n=31) matched for age (2-18 years), gender and socio-economic status were recruited. The supplemented group received one (2-4 years old), two (5-10), three (11-16) and four (≥ 17) omega 3 fatty acid capsule containing 277.8 mg DHA and 39.0 mg EPA. Renal function parameters were analysed in plasma and urine.

Results: The omega-3 group compared with the control had increased plasma urea nitrogen (2.5 ±0.8 vs 2.1± 0.7 mmol/l, p<0.05) and reduced urine albumin (96.6%, 0-200 mg/l(null); 3.7%, 300 mg/l; 0.0%, 1000 mg/l vs 75.0%, 0-200 mg/l (null); 21.9%, 300 mg/l; 3.1%, 1000 mg/l, p<0.01). Supplementation had no effect on plasma creatinine (3.2 ± 1.1 vs 3.3 ± 0.9 mg/l , p>0.05), potassium (4.1 ± 0.6 vs 4.3 ± 0.8 mmol/l, p>0.05), sodium (136.7 ± 8.9 vs 137.1 ± 9.2 mmol/l, p>0.05) and calcium (91.0 ± 6.0 vs 92.0 ± 13.0 mg/l, p>0.05), urine specific gravity (1.01 ± 0.002 vs 1.01 ± 0.002, p> 0.05) and urine RBC count under high power field (1.03± 0.18 vs 1.09 ± 0.39, p>0.05).

Conclusion: The results of this pilot study provide limited evidence of improved renal function in SCD patients supplemented with DHA and EPA. A well-powered further study is needed.

n-3 polyunsaturated-rich diets during the perinatal period alter the fatty acid profile and status of Zn and Fe in the hippocampus with impact on locomotor activity, memory and depression in the offspring

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Long chain n-3 fatty acids (n-3 LC-PUFA) play a role in behavioral and cognitive functions, and may be effective in the treatment of depressive disorder. We investigated whether fish and linseed oils intake, rich in PUFA n-3, during the perinatal period affect the aversive, spatial and recognition memory, depression, odor discrimination, exploratory and locomotor activity, and associate with hippocampal fatty acid (FA) profile and the status of Zn and Fe. Rats were fed with isocaloric semisynthetic diets containing 7% of non-vitamin fat component based on either soybean- (S), linseed- (L) or fish-oil (F) during pregnancy and lactation and for the post-weaning male pups up to 49 days age. The pups attaining 30 days age underwent several behavioral tests and at 49 days were decapitated. The hippocampus were dissected and analyzed by gas chromatography for the FA profile and Total Reflection X-Ray Fluorescence was used for Zn and Fe analysis. L and F groups presented significantly less immobility time in forced swimming and tail suspension tests, but L rats presented climbing behavior exacerbated. Decreased latency in Morris water maze was observed for the F group. In contrast, the locomotor and exploratory activity in open field test appeared to be the highest one in the L group as compared to the S group. There was a significant increase in objects recognition memory only for the F group. No change in aversive memory was observed between the groups. L hippocampus had a lower proportion of AA, addition of n-6 FA, DPAn-3, AA/DHA and increased Zn and Fe concentrations. In F hippocampus had the lowest values of linoleic acid, AA, alpha-linolenic acid, DPAn-3, C18:2n-6+C18:3n-3, n-6/n-3, AA/DHA, Zn and Fe. In conclusion, n-3 PUFA-rich diets influenced offspring hippocampus FA profile, Zn and Fe status with improved depression behavior and memory. However, hyperactivity was observed in L young rats.

Functional capillary density and inflammatory status in adult offsprings of dams fed with trans and interesterified fats

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Introduction: Recent reports have shown that excess fat in the diet or the type of fatty acid consumed by rats during pregnancy and lactation can result in cardiovascular dysfunction in adult offspring. The association between the intake of hydrogenated fat, rich in trans fatty acids (AGT), and the development of diseases to expose the existent public politics for the reduction of the intake of AGT. The food industry has been making possible alternatives as the interesterification to reduce AGT of the foods. However, there is a lack of studies of this lipid source on human health. Objective: To evaluate the impact of maternal intake of interesterified fat during gestation and lactation in vascular function and microcirculation in adult pups. Methods: We used adult female black C57BL/6 mice during gestation and lactation periods, the mice were divided in four groups each one receiving a diet made with a different fat source: control (soybean oil; CON), palm oil (PALM), trans fat (TRANS) and interesterified fat (INTER) groups. After weaning, the pups were accompanied till 90 days. Functional capillary density in brain (pia mater membrane) and skeletal muscle (gracilis muscle) was determined by intravital fluorescence microscopy in anesthetized animals (pentobarbital: 75mg/Kg, ip), intubated, immobilized, catheterized and artificially ventilated. The inflammatory status was evaluated by the observation of leukocyte-endothelial cell interactions in postcapillary venules. Results were considered significantly different when $p < 0.05$. Results: There was no difference in functional capillary density in brain and skeletal muscle. TRANS and INTER groups had greater number of rolling leukocytes compared to CON and PALM groups. Adhered leukocytes were higher in TRANS compared to all the others groups. Conclusion: Maternal intake of trans and interesterified fats are able to alter inflammatory status in adult offspring.

Palm Oil and Biodiversity in Amazon Area Represented by Wide Differences in Fatty Acid Composition

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Introduction: African Palm (Elaeis guineensis), whose principal producer is Malaysia, is one of the most important sources of vegetable oil in the world, presenting high economic importance. In South America, especially in the Amazon region, there are more than 700 species of palms, which could provide alternative sources of oil for human consumption in the future. The aim of this study was to determine the fatty acid profile of oils extracted from five different Amazon palm species. Method: Ripe fruits of the studied species were collected and classified by a botanic specialist: Bacuri (Attalea phalerata), Patauá (Oenocarpus bataua), Macaúba (Acrocomia aculeatae), Açaí (Euterpe precatoria) and Babaçu (Attalea speciosa). Their oils were extracted by cold pressing; and fatty acid methyl esters were prepared using ISO 5509 adding TG 13:0 as internal standard, being analyzed by gas-liquid chromatography. Results: The studied oils presented significantly different fatty acid profiles compared to Elaeis guineensis, whose main fatty acids are palmitic (48%) and oleic (40%) acids. Patauá presented the highest oleic (75%) and the lowest palmitic acid amounts (9%); Açaí showed 58% oleic and 18% palmitic acids. Macaúba presented 55% oleic and 18% palmitic acids; Patauá had the most balanced distribution between these two fatty acids, 38 and 26% respectively. Babaçu showed a very particular composition, with 36% Lauric acid, 20% Oleic acid, 9% palmitic acid, and a low percentage of Linoleic acid (0,6 - 2%). Conclusions: The studied Palm oils from Amazon region presented different fatty acid compositions compared to the African Palm. Four of those species presented good nutritional characteristics, with high oleic acid content combined with low palmitic acid content.

Dietary supplementation of trans-11 vaccenic acid reduces adipocyte size but neither aggravates nor attenuates obesity-mediated insulin resistance in fa/fa Zucker rats.

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Conjugated linoleic acid (CLA) present in dairy and ruminant fat has beneficial effects on metabolic syndrome characteristics. Production practices to increase milk content of CLA are also substantially elevating trans-11 vaccenic acid (TVA) in milk and dairy products. To date, research on the potential effects of TVA on human health is limited. Consequently, questions are being raised whether TVA has the same beneficial actions as CLA or has adverse biological effects like industrially produced trans fatty acids. Our study examined the effect of TVA on obesity-mediated metabolic abnormalities. Five week old fa/fa Zucker male rats (n=10/group) were randomly assigned to the TVA group (1.5% (w/w) TVA) or the control group (0% TVA) for 8 weeks. Dietary supplementation of TVA did not alter weight gain, feed intake, blood pressure or organ/body weight ratios except for a lower liver/body weight ratio in the TVA group compared to controls (3.73 ± 0.08 vs 3.93 ± 0.17 g/100g; $P<0.05$). However, the total liver lipid concentration, an indicator of hepatic steatosis, was not different between the groups. Likewise, there were no changes in fasting glycemia and lipidemia, or oral glucose tolerance. Although there were no physiological differences observed between groups, animals supplemented with TVA had smaller adipocytes (~10% decrease vs control). The TVA group also had higher adipophilin and interleukin-10 protein levels in epididymal adipose tissue (1.7 and 1.4-fold increase from control, respectively), however, there were no changes observed in critical nodes of insulin signalling. In summary, our study provides evidence that a naturally produced trans fat (TVA) does not have negative effects on obesity mediated-metabolic abnormalities.

DHA-labeled fluorophore is sensitive to membrane phase behavior and ordered domains

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Docosahexaenoic acid (DHA), a bioactive n-3 polyunsaturated fatty acid found in fish oil, exerts functional effects by targeting multiple mechanisms including manipulating membrane biophysical properties. A key limitation in the field is the lack of a fluorescently labeled DHA probe for studying membrane-based mechanisms. Here we first investigated if a new DHA-Bodipy probe, compared to a control palmitic acid (PA)-Bodipy probe, could report on long-term uptake of the fatty acid into polar lipids and raft-like domains of EL4 cells. Biochemical analysis and live cell imaging revealed DHA- and PA-Bodipy did not efficiently incorporate into polar lipids or into detergent resistant raft-like membranes; instead, the probes differentially localized into the mitochondria and endoplasmic reticulum. Next, we investigated if DHA-Bodipy was sensitive to changes in membrane phase behavior. Quantitative analysis of time-lapse movies revealed DHA-Bodipy was equally as sensitive to membrane phase behavior as PA-Bodipy. We then investigated if the DHA-Bodipy was more sensitive to ordered raft-like versus disordered non-raft like membrane domains. To accomplish this, we employed time-resolved fluorescence anisotropy measurements in lipid vesicles of controlled composition. Both probes were sensitive to formation of ordered and disordered domains; however, in ordered domains, DHA-Bodipy displayed decreased molecular order than PA-Bodipy. Altogether, DHA-Bodipy did not effectively report on long-term uptake into polar lipids, but was highly sensitive to membrane phase organization and revealed novel insight into DHA's molecular motional organization in an ordered raft-like domain environment.

Lycopene Modulates Inflammatory Cytokines Production and Treg Cell Population in Mitogen-Activated Peripheral Mononuclear Cells

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Background: Epidemiological evidence shows a negative association between dietary lycopene, a carotenoid found mainly in tomato-based foods, and cardiovascular disease (CVD) risk. Lycopene may possess anti-inflammatory properties potentially responsible for its beneficial effect observed on CVD risk.

We previously showed that lycopene influences lymphocyte proliferation through mechanisms dependent on processes involved in early cellular activation.

Objective: The aim of the study was to determine the effects of lycopene in-vitro on mitogen-stimulated T lymphocyte cytokine production in relation to Th1/Th2 and Treg responses.

Procedures: Peripheral mononuclear cells were isolated from 16 healthy adults and cultured for 18h, 36h and 60h in the presence of lycopene-enriched liposomes (0-1.18µg lycopene/ml) with or without Concanavalin A (15µg/ml) or anti-CD3 (0.5µg/ml). Liposomes without lycopene were used as positive control. Cytokines were measured by ELISA (IL-1-b, IL-2, IL-10, IFN-g, TGF-b) or cytometric bead array (IL-4, IL-10, IL-17A, IFN-g). The population profile of Treg (CD4+ CD25+) and subsets, nTreg (CD4+ CD25+FoxP3+) and iTreg (CD4+ CD25+ IL-10+) was determined by flow cytometry.

Results: Lycopene significantly increased the Treg population by 36% whilst increasing the proportion of iTreg but decreasing the proportion of nTreg. Lycopene also significantly inhibited early production of both Th1 and Th2 associated cytokines (IL-2, IFN-g and IL-10). After 36h and 60h, IL-10, IL-17 and IFN-g were significantly decreased while lycopene had no effect on IL-4 and TGF-b production.

Conclusion: Low concentrations of Lycopene increased Treg cells while inhibiting mitogen-induced cytokine production but not specifically towards a Th1 or Th2 related response. These data indicate that lycopene could be beneficial against CVD by modulating the inflammatory process involved in atheromatous plaque formation.

Maternal plasma HDL cholesterol level and childhood overweight: the KOALA Birth Cohort Study

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Background and aims - Overweight and obesity may already be programmed during foetal life. Relations are known between maternal and childhood BMI, as well as between maternal and childhood serum lipids. We evaluated whether high maternal total and HDL-cholesterol is associated with the child's risk of overweight, and whether this is independent from maternal BMI.

Material and methods - The study included 1303 mother-child pairs from the KOALA Birth Cohort Study (www.koala-study.nl) with maternal blood collected at 36 weeks of pregnancy. During follow-up data were collected by questionnaires and measurements including children's weight and height at ages 1, 2, 4-5 and 6-7 years. Outcomes were the children's BMI and overweight (using age dependent cut-off points for BMI). Statistical analyses with Generalized Estimation Equations adjusted for multiple confounders, including breastfeeding.

Results - 145 (11%) children developed overweight between birth and age 7. Higher maternal HDL was associated with a lower risk of child's overweight (P-value for decreasing odds ratio over quintiles P=0.015, odds ratio 0.56 (95% confidence interval 0.32-1.01) for the highest vs lowest quintile of HDL). This association was independent of maternal pre-pregnant BMI and maternal weight gain during pregnancy.

Conclusions - High maternal HDL-cholesterol is associated with lower risk of overweight in the offspring, and this is not solely explained by maternal overweight. Apart from intrauterine programming also shared genetic variants and nutrition may be implicated.

Age-related decrease in hippocampal neural stem/progenitor cells is ameliorated by arachidonic acid ingestion in rats

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Background: Hippocampal neurogenesis is related to learning and memory, and the number of neural stem/progenitor cells (NSPCs) and newborn neurons (NNs) decreases with age. Docosahexaenoic acid

(DHA) ingestion has been reported to increase NNs in aged rats deficient in n-3 fatty acids. However, effects of arachidonic acid (ARA) and/or DHA, the main components in hippocampal fatty acids, on NSPCs and NNs remain unclear in normal aged rats.

Objective: To examine effects of successive ingestion of ARA and/or DHA on age-related decrease in NSPCs or NNs in rats.

Procedure: Male F344 rats were fed modified AIN-76A diet containing ARA and/or DHA (0.2% in diets, respectively) from 2 to 18 months old, and sacrificed 1 day or 4 weeks after 5-bromo-2-deoxyuridine (BrdU) injections at 2, 6 and 18 months. The number of NSPCs (SOX2+/BrdU+) and NNs (NeuN+/BrdU+) was evaluated immunohistochemically.

Results: Total BrdU+ cells at 1 day after injections were decreased to 63% (6 months) and 11% (18 months) against those at 2 months. The number of total BrdU+ cells in ARA-ingested aged rats was 165% larger compared to control, while it was not affected by DHA ingestion. The ratio of SOX2+ cells against BrdU+ cells was unchanged by aging nor by ARA or DHA ingestion. NeuN+/BrdU+ cells at 4 weeks after BrdU injections were decreased to 40% (at 6 months) and 7% (at 18 months) against those at 2 months, but increased to 134% in DHA-ingested aged rats compared to control (not statistically significant, though).

Conclusion: These results indicate that ARA ingestion can ameliorate the age-related decrease in the number of hippocampal NSPCs. Moreover, functions of ARA and DHA on hippocampal neurogenesis seem to be differential in aged rats; ARA may maintain a pool of NSPCs, while DHA may sustain production of NNs.

The anticonvulsant properties of docosahexaenoic acid in rodents

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Introduction: Epilepsy is a neurological disorder which is defined by spontaneous and recurrent seizures and affects approximately 1% of the population. The current treatment of choice for epilepsy are antiepileptic drugs (AED). However, 20 to 40% of patients aren't fully controlled or controlled at all. Therefore, alternative treatments are still needed. Growing evidence suggests that DHA has anticonvulsant properties.

Objective: We explored the possibility that DHA has anticonvulsant properties when administered chronically, sub-chronically, and acutely.

Methods: For the chronic study, rats were implanted with bipolar electrodes in the amygdala. Following two baseline measurements of afterdischarge thresholds (ADT), animals were randomized into either fish oil (40% of fat content) or control (soybean oil). ADT were measured every month for 8 months.

For the sub-chronic study, rats received 50mg/kg i.p. of either unesterified DHA, DHA ethyl ester (DHA EE) or saline for 14 consecutive days. On day 15, rats were seizure tested with pentylenetetrazole (PTZ) and the latency to tonic-clonic seizure was measured.

For the acute study, rats were infused i.v. with either 0, 12.5, 25, 50, 100, or 200mg/kg of unesterified DHA over 5min. After 5min, animals were tested with PTZ and latency to tonic-clonic seizure was measured.

Results: In the chronic study, ADT in the control group dropped while ADT in the fish oil group remained at baseline. A significant interaction between time and treatment was measured ($p < 0.05$) with a significant difference between control and fish oil groups at month 3, 5, 7 ($p < 0.05$)

Fourteen consecutive days of DHA and DHA EE i.p. administration increased seizure latency in the maximal PTZ model ($p < 0.05$) while a 5 minute i.v. infusion of unesterified DHA also increased seizure latency ($p < 0.05$), albeit no dose-response relationship was observed.

Conclusion: These results suggest that DHA may have antiepileptic properties, providing a potentially cheap therapy for epilepsy.

Barramundi has limited ability to convert dietary ALA to omega-3 long chain polyunsaturated fatty acid

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Substitution of marine-derived oils and protein with alternative sources without compromising fish health and product quality is urgently needed to eliminate the heavy dependence on marine fisheries. This study determined the effects of substituting fish oil and fish meal in the diet with a blend of vegetable oils and defatted poultry meal on barramundi fillet and liver fatty acid profiles. The dietary treatments consisted of vegetable oil-based diets with an ALA content ranging from 0.1 to 3.2%en with the LA content held constant at 2.4%en. A commercial diet which contained fish-derived EPA and DHA was used as a reference group. Results showed that the fatty acid composition of fish liver and fillet reflected the dietary lipid source. As the ALA content of the diet increased, the ALA level in the liver and fillet increased in a dose-dependent manner. There was, however, no corresponding increase in the tissue levels of the n-3 LCPUFA EPA, DPA and DHA. Increasing levels of dietary ALA has no effect on hepatic mRNA expression of desaturase (FADS2) and elongase (ELOVL) genes. Hepatic gene expression levels of FADS2 and ELOVL were increased by approximately 10 fold and 3 fold, respectively, in all vegetable oil-based dietary groups relative to the fish oil-based reference diet. These data demonstrate that there may be a disconnection between gene expression and fatty acid status since dietary fatty acids altered expression but this had no effect on fatty acid levels. The large amount of variation between individual fish in their tissue n-3 LCPUFA content may point to the possibility of a selective breeding program.

Correlations between blood and tissue omega-3 LCPUFA status following dietary ALA intervention in rats

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There are relatively few reports available on the extent to which the fatty acid pattern of plasma or erythrocyte phospholipids correlates with the fatty acid pattern of tissue phospholipids after supplementing dietary polyunsaturated fatty acids (PUFA). To obtain a wide range of long chain (LC) PUFA we subjected weanling rats to dietary treatment with the omega-3 (n-3) LCPUFA precursor, alpha linolenic acid (ALA) for 3 weeks. With the exception of the brain, we found tight and consistent correlations between the total n-3 LCPUFA fatty acid content of both plasma and erythrocyte phospholipids with fatty acid levels in all tissues (r=0.92-0.99, P<0.0001 in all cases). The relationships between eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) content in blood and levels in liver, kidney, heart and quadriceps muscle phospholipids were stronger than those for docosahexaenoic acid (DHA). The strong correlations between the EPA+DHA (the Omega-3 Index), total n-3 LCPUFA and total n-3 PUFA contents in both plasma and erythrocyte phospholipids and tissues investigated in this study suggest that, under a wide range of n-3 LCPUFA values, plasma and erythrocyte n-3 fatty acid content reflect accumulation of endogenously synthesized n-3 LCPUFA, and thus can be used as a reliable surrogate for assessing n-3 status in tissues. This study also highlights the need to exercise caution when using blood as marker of fatty acid levels in the brain following short-term dietary interventions.

Covalent adduct hybrid chemical ionization (CAHCI) mass spectrometry for high sensitivity structural analysis of fatty acid methyl esters

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Acetonitrile covalent adduct chemical ionization tandem mass spectrometry (CACI MS/MS) is a convenient and unambiguous method for identifying fatty acid methyl ester (FAME) double bond positions

using a gas chromatograph (GC) coupled to an ion trap mass spectrometer. When the ion trap is operated in internal ionization mode, the CI gas is ionized in the ion trap and an acetonitrile derived reagent ion (m/z 54) reacts across the double bonds of FAME, forming the [M+54]+ adduct ions. In CACI MS/MS, [M+54]+ ions are isolated and fragmented, yielding strong diagnostic ions that enable identification of double bond positions. A limitation of internal ionization CACI-MS/MS is that the ion trap stores a finite number of ions per cycle. The formation of [M+54]+ adduct ions is limited by the presence of other, higher abundance products of acetonitrile ionization (e.g. m/z 42) relative to the m/z 54 ions. In covalent adduct hybrid chemical ionization (CAHCI) MS, the CI gas is ionized in an external chamber and only the selected reagent ions (m/z 54) are stored in the ion trap and allowed to react with FAME. Analysis of a FAME reference standard showed [M+54]+ ion intensities >20x greater than those obtained by CACI MS, and sensitivity was particularly enhanced for highly unsaturated FAME (e.g. 22:6n-3). CAHCI MS/MS spectra showed strong diagnostic ions and fewer interfering ions compared with CACI-MS/MS. These results demonstrate that CAHCI greatly enhanced the sensitivity and selectivity of diagnostic ions for the identification of FAME double bond positions. The enhanced formation of [M+54]+ ions from highly unsaturated FAME indicates a major advantage of CAHCI MS/MS over CACI MS/MS for the identification of low-abundance conjugated and/or non methylene interrupted polyunsaturated FAME.

Prostamides PGE2-EA and PGF2a-EA are found in rabbit cornea

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One main function of the cornea is to protect the intraocular structures of the eye. It does this partly by responding to intraocular pressure. Corneal injury resulting from scratching, surgery or infections, can cause pain and result in tissue scarring. Injury stimulates arachidonic acid release, cyclo-oxygenase-2 (COX-2) up-regulation and, consequently, formation of bioactive lipid mediators including prostaglandins (PG). PGE2 suppresses the mitogenic response to epithelial growth factor, thus regulating cell proliferation which, if left unchecked, can result in scarring, whilst PGF2a is a well established anti-hypertensive agent. Stable analogues of prostaglandins such as bimatoprost, an ethanolamide derivative of PGF2a, are widely used in the treatment of glaucoma and management of ocular hypertension.

Here, we report the identification of prostaglandin ethanolamides (prostamides; PG-EA) PGE2-EA and PGF2a-EA in rabbit corneal tissue. Lipid extracts were analysed and quantified by liquid chromatography coupled to electrospray ionisation tandem mass spectrometry (LC/ESI-MS/MS).

The concentrations of PGE2-EA and PGF2a-EA were 0.02 and <0.01 pg/mg tissue, respectively. The ability of the corneal tissue to metabolise anandamide (A-EA) to prostamides via COX-2, was confirmed using tissue homogenate incubated with A-EA (10microM). This generated PGE2-EA and PGF2a-EA, at a ratio of 3:1. Profiling of corneal prostaglandins revealed production of PGE2 and PGF2a at the same ratio, suggesting that the profile of prostamides is also determined by the expression of the corresponding prostaglandin synthases. Furthermore, analysis of A-EA (A-EA, 0.55 pg/mg) and 6 other N-acyl-ethanolamide congeners (0.03-1.39 pg/mg tissue) found in rabbit cornea, revealed that A-EA is a relatively minor product therefore explaining the low concentration of prostamides found.

Overall, the identification of prostamides in corneal tissue confirms the presence of this family of lipid mediators in the eye and may, in part, explain the potent pharmacological activity of prostaglandin derivatives in ocular health.

Dietary n-3 polyunsaturated fatty acids reduce allergic sensitization in a mouse model for cow's milk allergy

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Cow's milk allergy is the most common food allergy in children and no effective treatment is available. Long chain n-3 polyunsaturated fatty acids (n-3 LCPUFA) may prevent allergic disease. Aim of this study was to assess whether dietary supplementation with n-3 LCPUFA prevents the establishment of food

allergy. C3H/HeOuJ mice were fed a 4% soy oil/6% tuna oil diet rich in n-3 LCPUFA or a control diet (10% soy oil, high in n-6 PUFA) before and during oral sensitization with whey, using cholera toxin as an adjuvant. The acute allergic skin response (ear swelling), red blood cell membrane lipid composition, serum immunoglobulins and mouse mast cell protease-1 (mMCP-1) and percentages regulatory T-cells (Treg) in spleen and small intestine were assessed. The tuna diet enhanced the n-3 LCPUFA content in RBC membranes, while reducing n-6 LCPUFA. The acute allergic skin response was reduced by over 50% in sensitized animals fed the tuna diet as compared to the control diet (p<0.001). In addition, whey-specific IgG1 levels were decreased in the tuna diet group (p<0.05), IgE showed the same tendency. Hence, the Th2 type humoral response was suppressed. Although the tuna diet did not reduce mMCP-1, sera of tuna diet fed sensitized mice had a diminished capacity to induce an allergic effector response in naive recipient mice compared to control sera (p<0.05) using serum transfer. In addition, the acute skin response was diminished in tuna diet fed naive recipient mice injected with whey hyperimmune serum. Furthermore, only whey sensitized mice fed the tuna diet had a higher percentage Treg in spleen and intestinal lamina propria as compared to sham animals (p<0.05). In short, dietary n-3 PUFA largely prevented allergic sensitization in a murine model for food allergy by suppressing the induction of the Th2 type B cell response and enhancing Treg percentages.

Dietary n-3 LCPUFA reduce blood pressure and improve endothelial function in spontaneously hypertensive rats

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Hypertension is associated with high cardiovascular morbidity and mortality and involves endothelial dysfunction, comprising reduced relaxation potential and enhanced contractility of the vasculature. There is great interest in novel therapies, including dietary interventions, to treat this disease state. N-3 LCPUFA from oily fish are known to exert beneficial effects in cardiovascular homeostasis. Aim of this study was to investigate the effect of dietary n-3 LCPUFA on blood pressure and endothelial function in spontaneously hypertensive rats (SHR). 12-week-old SHR were fed a 3% soy oil/4% tuna oil diet or control diet during 12 weeks, after which blood pressure and ex vivo carotid artery endothelial function were determined. Intra-arterial blood pressure measurements showed that systolic (193 ± 11 vs 168 ± 7), diastolic (165 ± 13 vs 146 ± 13) and mean arterial pressure (174 ± 8 vs 153 ± 8) were significantly reduced by 12 weeks of fish oil treatment (p<0.01), while heart rate was not affected. These results were confirmed by tail cuff measurements in awake animals. Endothelial function was restored by fish oil, as indicated by an improved relaxation potential towards methacholine (91.7 ± 3.6 vs 72.1 ± 12.3% relaxation, p<0.001) as compared to control diet fed rats. Concomitantly, endothelium-dependent contractility at higher methacholine concentrations was reduced (p<0.05). However, the contractile response to the thromboxane analogue U46,619 was not different. In addition, we have previously shown that hypertension is associated with marked alterations in sphingolipid biology, such as strongly increased endothelium-dependent contractile responses to exogenous sphingomyelinase (Spijkers, et al. 2011). In the present study the carotid artery response to sphingomyelinase tended to be reduced in n-3 LCPUFA fed rats. In conclusion, dietary n-3 LCPUFA reduce blood pressure and improve endothelial function in spontaneously hypertensive rats.

Omega-6 fatty acid status and current suicide risk in early-pregnancy: A prospective cohort study of low-income women of Rio de Janeiro, Brazil

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Background: Anxiety and depression are risk factors for suicide and are common in pregnancy. Here we estimate the prevalence of women with current suicide risk during the first trimester of pregnancy and determinate whether fatty acids serum compositions are associated with increased suicide risk.

Methods: Cross-sectional analysis of 245 pregnant women enrolled on a prospective cohort study based on a prenatal care public health center in Rio de Janeiro, Brazil. Eligibility criteria included: being between the 6th and 13th gestational week, aged 20-40 years, free from chronic or infectious diseases, and

singleton pregnancy. Baseline interviews included a psychiatric assessment; the dependent variable, current suicide risk (yes/no) was defined by the M.I.N.I International Neuropsychiatric Interview (DSM-IV; version 5.0.0; score: 0=absent; 1-5=low; 6-10=moderate; >=10=high). Fatty acids composition was determined in serum samples obtained before 13th week, and assayed utilizing a high-throughput robotic direct methylation coupled with fast gas-liquid chromatography. Statistical analyses included the Kruskal-Wallis test, univariate and multivariate logistic regressions. Fatty acids data were expressed as percent of total fatty acids and converted to Z scores and entered as continuous variable into logistic regression analysis models.

Results: Prevalence of any current suicide risk, defined by the MINI, was 19.5%, with 5.3% meeting criteria for high risk. Higher likelihood of any suicide risk was observed among women with higher arachidonic acid [ARA (20:4n-6): OR=1.58, 95% CI 1.10-2.27,p<0.013] and gamma-linolenic acid [GLA (18:3 n-6): OR=1.41, 95% CI 1.01-1.98,p<0.042] levels, per SD of fatty acid, in adjusted logistic regressions. Median blood levels of both fatty acids [mean(range)] were higher according to level of suicide risk (absent, low or moderate, high risk) respectively: GLA=0.30(0.08-0.95) vs. 0.32(0.15-0.81) vs. 0.41(0.28-0.66), p<0.006; ARA=9.15(4.07-14.01) vs. 9.03(5.18-13.04) vs. 10.19(0.74-13.57),p<0.09).

Conclusion: Higher arachidonic and gamma-linolenic acid status was associated with greater likelihood of current suicide risk among low income pregnant Brazilian women.

Farmed Tilapia Are Good Source of Linoleic Acid (LA) But Not of Docosahexaenoic (DHA) or Eicosapentaenoic (EPA) Acid

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Background: Tilapias are widely farmed freshwater fish because of their adaptability to diverse environmental temperatures, fast growth rate and reproduction at high density in captivity. Although tilapias are native to most of Africa and the middles east, the Nile tilapias, locally known as Bulti, are the most widely farmed, and sold as a good source of omega 3 fatty acids in European and Asian food markets.

Objective: To investigate whether farmed tilapias (a) retain omega 6 and 3 composition of their wild ancestor, the Nile Bulti; (b) are good source of omega 3 fatty acids, particularly EPA and DHA.

Procedure: Wild, Nile Bulti, tilapias from White, Blue and Main Niles (Sudan) and farmed tilapias, fresh from Beijing (China) and frozen from China and Zimbabwe imported to the UK markets, were collected. Muscle samples were homogenised and analysed by the conventional technique for fatty acids.

Results: The frozen and fresh farmed tilapias had comparable saturated, monounsaturated, omega 6, omega 3 fatty acid composition. In contrast, there were remarkable differences in the levels of some fatty acids between the wild Nile Bulti and farmed tilapias. The farmed tilapias had higher oleic (27.7±0.4 vs 8.5±0.7, p<0.0001), LA (17.5±0.9 vs 6.0±0.5, p<0.0001), monounsaturated (36.2±0.6 vs 16.7±1.1, p<0.0001) and total omega 6 (23.4±0.9 vs 19.2±1.2, p<0.01) compared with their wild counterparts. However, they had lower alpha-linolenic (1.5±0.1 vs 2.7±0.4, p<0.01), EPA (0.26±0.03 vs 3.5±0.3, p<0.0001), omega 3 DPA (0.98±0.08 vs 6.4±0.2, p<0.0001), DHA (3.0±0.2 vs 13.5± 0.6, p<0.0001), total omega 3 (6.0±0.3 vs 26.9±1.0, p<0.0001), total omega 3/omega 6 ratio (0.26±0.02 vs 1.5±0.1, p<0.0001) and arachidonic (1.7±0.09 vs 8.5±0.7, p<0.0001).

Conclusion: Contrary to the marketing claims, farmed tilapias, unlike their wild counterparts, are not good source of EPA, DHA or total omega 3. However, like most vegetable seed oils, they could be good source linoleic acid. The current fish farming practice leads to a drastic change in fatty acid composition of tilapias. Hence, there may be a need to re-assess the farming practice so that they could retain their original (natural) composition.

The Nile Fish Species, Bulti, Dabs and Gargour, Contain Adequate Amounts of DHA and AA To Help Support Pregnancy and Lactation

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Background: A significant number of expectant and nursing Sudanese women claim to eat regularly fresh water fish from the Nile River. However, maternal and neonatal blood and breast milk from Sudan have very low levels of DHA. As fresh water fish are thought to contain significant amounts of omega 3 fatty acids, the sub-optimal DHA status of Sudanese women is rather intriguing. The fresh water fish, Bulti (Oreochromis niloticus niloticus), Dabs (Labeo niloticus) and Gargur (Synodontis clarias) found in Blue, White Niles, are commonly consumed in Sudan.

Objective: To assess omega 3 and 6 fatty acid contents of the popularly-eaten and relatively inexpensive Nile water species, Bulti, Dabs and Gargour.

Procedure: Bulti (Oreochromis niloticus niloticus), Dabs (Labeo niloticus), Gargur (Synodontis clarias) fish were collected from the Blue, White and Main Niles about 30 kilometre distance from the confluence point and upstream of the confluence. Representative muscle samples were homogenised and analysed for fatty acids with GC-MS.

Results: Dabs had higher percent of EPA (5.3 – 7.6), DHA (13.6 – 18.4) and total omega 3 (23.2-31.0) compared with Bulti (2.6 – 4.5), (12.4 – 15.5) and (21.2 – 26.4) and Gargur (2.5 – 4.6), (8.7 – 11.8) and (14.5 – 21.2). Arachidonic acid level was higher in Bulti (7.0 – 10.3) and Dabs (7.0 – 11.6) than in Gargur (5.3 – 6.8). Both Dabs (1.9-3.0) and Gargur (2.6 – 4.4) had lower levels of linoleic compared with Bulti (5.3 – 6.8). The White Nile Dabs compared with their counterparts from the Main and Blue Niles had higher percent DHA (18.3±0.9 vs 14.1±.1 vs 13.6 ±0.7, p<0.01), total omega 3 (31.0±1 vs 24.7±3.0 vs 23.2±1.7, p<0.05) and AA (11.6±0.5 vs 7.0±1.1 vs 7.1±0.9, p<0.01). In contrast, the levels of DHA and total omega 3 were significantly (p<0.05) higher in Bulti from the Main Nile (15.5±0.2 and 26.4±0.1) than those collected from White (12.7±1.5 and 24.8±2.8) and Blue (12.4±0.6 and 21.2±0.8). Blue Nile Gargur had the lowest level of DHA and total omega 3 (8.7±0.4 and 14.5±0.7) compared with White (11.1±2.6 and 17.7±3.6) and Main (11.8±1.7 and 21.2±1.8) Nile Gargur (p<0.05).

Conclusion: These three fish species commonly consumed in Sudan, which seem to contain DHA percent broadly comparable to that of cod liver oil, and AA would be expected to provide adequate amounts of both nutrients for expectant and nursing mothers.

Habitual intake of dietary total and n-3 polyunsaturated fatty acids are associated with 24-hour ambulatory blood pressure in a population with increased risk of cardiovascular disease

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The amount and composition of fats in the diet have been implicated in the aetiology of cardiovascular disease (CVD). Blood pressure, a key component of vascular function, is an important risk factor for CVD morbidity and mortality, with hypertension an important target for dietary modifications. The aim of this study was to investigate the associations between habitual dietary fatty acid intake and 24-hour ambulatory blood pressure in men (n=50) and women (n=72) with an increased risk of CVD. A scoring system based on fasting lipids, blood pressure, BMI and family history of CVD was used to recruit the adults (n=122, mean (SD) age 43 (10) years and body mass index 27.0 (4.1) kg/m2), with a score of ≥ 2 associated with CVD risk. Habitual dietary intake was assessed by completion of 4-day weighed food diaries which were analysed using Dietplan 6.6. Ambulatory blood pressure monitors (ScanMed Medical) recorded systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure and heart rate (HR) for a 24-hour period, every 30 minutes during the day and hourly during the night. Digital Volume Pulse was additionally measured by photoplethysmography, as an index of large arterial stiffness. There were significant negative correlations between 24-hour SBP and total dietary %E PUFA (Pearson's correlation coefficient (r) =-0.195, P=0.031) and %E n-3 PUFA (r=-0.205, P=0.023). Neither the intake of total fat nor the intake of saturated or monounsaturated fatty acids were associated with mean 24-hour, day or night SBP, DBP, pulse pressure and HR. There were no correlations between the intakes of the

dietary fatty acids and arterial stiffness. In conclusion, higher dietary total and n-3 PUFA intakes were associated with lower 24-hour SBP in a population with an increased risk of CVD.

Post-prandial incorporation of marine omega-3 fatty acids into plasma triacylglycerol and NEFA when consumed in different chemical forms

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Study Aim: To investigate whether different chemical forms of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (triacylglycerol (TG), ethyl ester (EE), free fatty acids (FFA)) are equally available and incorporated into human plasma triacylglycerol and NEFA pools in the post-prandial period.

Background: EPA and DHA may be found in seafood and in supplements in different structural forms. From all of the available literature, it is not clear as to whether EPA and DHA are equally available to the human body when presented in these different chemical forms.

Study Design: Healthy male volunteers (n 10) aged 18-40 years were recruited into a double blind cross-over trial. Each volunteer consumed EPA and DHA in capsules, and in different chemical forms (TG, FFA and EE); all volunteers consumed all chemical forms in a predetermined random order. All supplements used had the same ratio and amount of EPA and DHA (1.8 g). Volunteers were cannulated in the fasting state and blood was collected at baseline; volunteers then consumed a standard breakfast and the capsules and blood was collected at 9 time points over 6 hours. Plasma was isolated at all time points. All 10 volunteers took part in all post-prandial study days, which were at least 14 days apart. Incorporation of EPA and DHA into plasma triacylglycerol and NEFA fractions was measured using gas chromatography.

Results: There were no significant differences between chemical forms with regard to the appearance of EPA and DHA into plasma triacylglyerol or NEFA over the 6 hour post-prandial period.

Conclusions: From this data, EPA and DHA presented to the human body in different chemical forms appear to be equally bioavailable. The phospholipid form was not examined.

Tissue arachidonic acid content is influenced by dietary g-linolenic acid and arachidonic acid, but not linoleic acid

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Linoleic acid is the most highly consumed polyunsaturated fatty acid in the Western diet and is found in virtually all commonly consumed foods. The concern with dietary linoleic acid, being the metabolic precursor of arachidonic acid, is its consumption may enrich tissues with arachidonic acid and contribute to chronic and overproduction of bioactive eicosanoids. As such, recent reviews have recommended limiting linoleic acid intake as an effective way to reduce tissue arachidonic acid levels. However, no systematic review of human trials regarding linoleic acid consumption and it metabolic derivatives on subsequent changes in tissue levels of arachidonic acid has been undertaken. In this study, we reviewed the human literature that reported changes in dietary n-6 polyunsaturated fatty acids, including linoleic acid and its subsequent impact on changing arachidonic acid content in the phospholipid pool of erythrocytes and plasma/serum. We reviewed the published literature presenting data outlining changes in dietary linoleic, g-linolenic and arachidonic acids in adult human clinical trials that reported changes in plasma/serum and erythrocytes phospholipid arachidonic acid content in adults consuming Western-like diets. Increasing or decreasing dietary linoleic acid levels were not significantly correlated with changes in arachidonic acid levels in the phospholipid pool of plasma/serum or erythrocytes. However, there was a positive relationship between dietary g-linolenic acid and dietary arachidonic acid on changes in arachidonic levels in plasma/serum phospholipids. Our results suggest that consuming n-6 PUFA with 3 or more double bonds positively influence arachidonic acid content in the phospholipid pool of plasma/serum and erythrocytes, with null effects from linoleic acid in adults consuming Western-type diets.

Dose translation of dietary polyunsaturated fatty acids from rodents to humans

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As the foundation for preclinical research, the scientific community relies heavily on animal models as a key determinant for moving into human clinical trials. Through careful environmental control, these genetically similar models facilitate therapeutic advancements in the understanding and treatment of human disease. Currently, no guidelines exist for appropriate dosing of dietary PUFAs for experimental models as they relate to humans and their intakes. At issue is the following question, "How do we translate the dose of dietary PUFA from rodents to humans?". The ability to extrapolate biological effects of dietary PUFAs from rodents to humans necessitates an allometric scaling model that is rooted within a human equivalent context. As such, C57BL/6J mice were divided into 23 dietary groups fed a background diet equivalent to the US diet based on metabolic differences (i.e., % energy) and further supplemented with "human equivalent doses" of n-6 PUFA (LA, AA) and n-3 PUFA (ALA, EPA). Changes in phospholipid fatty acid compositions were monitored in serum/plasma and erythrocytes and compared to data in the scientific literature from humans supplemented with equivalent doses. Increasing dietary LA had little effect on tissue AA, while supplementing diets with AA significantly increased tissue AA levels, importantly recapitulating results from human trials. On the other hand, dietary ALA and EPA increased mouse EPA phospholipid levels to a greater extent than observed in humans (reflecting species differences in the metabolism of dietary n-3 PUFA), but had minimal impact on changing DHA levels (similar to humans). However, when the background diet of ALA increased from 0.7%en to 1.4%en, changes in tissue fatty acid content (following suplementation of ALA or EPA) were more consistent with the human data. In summary, translation of dietary PUFAs between species may be accomplished using a theoretical model for allometric scaling based on energy and metabolic differences.

The Effect of EPA/DHA Supplementation on Children with Attention-Deficit / Hyperactivity Disorder. A Randomized Double-Blind, Placebo-Controlled Study

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Attention deficit / hyperactivity disorder (AD(H)D) is one of the most common developmental childhood disorders (worldwide prevalence rate 5.3%). The multifactorial and clinically heterogeneous disorder begins in early childhood and is characterized by inattention, hyperactivity and impulsivity. Although 70-85% of the patients are being treated successfully with stimulant medication, there is still demand for other treatment options.

For more than 20 years, long chain polyunsaturated fatty acids are being discussed as a therapy option for children with AD(H)D. However, the published results are ambiguous and further research efforts are necessary to clarify the role of LC-PUFAs as a possible treatment for children with AD(H)D.

Objective: To assess whether supplementation with EPA/DHA decreases symptoms in children diagnosed with AD(H)D according to DSM-IV criteria.

Methods: Study design: Randomized, double-blind, placebo-controlled study, 16 weeks intervention Inclusion criteria: Children 6-12 years, AD(H)D diagnosis according to DSM-IV criteria Exclusion criteria: Stimulant medication, use of omega-3 supplements during the previous 4 month, IQ ≤ 85

Outcome measures: Parent (DISYPS, CBCL) and teacher-rated (DISYPS, TRF) questionnaires Hamburg-Wechsler-Intelligence Test for Children; KITAP (Measure of attention: Testbatterie zur Aufmerksamkeits-prüfung; 6-12y); Erythrocyte fatty acid profiles; BDNF serum concentration

The following questions are addressed:

Does supplementation with EPA/DHA

1. affect behavior (teacher and parent-rated) and cognition (executive functions) of children diagnosed with AD(H)D?

2. affect composition of the erythrocyte fatty acid profile?

Do changes in fatty acid composition correlate with the observed behavioral and cognitive changes?

3. affect peripheral BDNF (Brain-Derived Neurotrophic Factor) levels?

Do BDNF level changes correlate with the observed behavioral and cognitive changes?

Preliminary data are presented

Enhancing cognitive functions in mild cognitive impairment with omega-3-fatty acid or resveratrol supplementation in combination with exercise and cognitive training - Proof of concept and mechanisms

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Background: Mild cognitive impairment (MCI) is viewed as a prodromal stage of Alzheimer's disease. Evidence from epidemiological studies suggests that high intake of fatty fish as well as regular physical and mental activity may protect against age-related cognitive decline. In addition, resveratrol has been shown to mimic caloric restriction, the most effective nutritional intervention for slowing aging. However, controlled intervention studies involving MCI patients, which investigated neuroprotective effects of such dietary and lifestyle interventions are lacking.

Objectives: To investigate the effect of dietary interventions (omega-3-fatty acid and resveratrol) and lifestyle interventions (physical activity and cognitive stimulation) on cognitive performance in MCI patients. The study will answer fundamental questions about intrinsic mechanisms of diet- and exercise-mediated effects on learning in the elderly impaired brain, including neurochemical pathways.

Methods: The first study will be carried out to test the efficacy of dietary interventions on functional and structural integrity of the brain. Participating subjects receive fish-oil (1320 mg EPA + 880 mg DHA/d), resveratrol (200 mg/d), or placebo (olive oil). Memory performance will be tested using different cognitive scales. Moreover, underlying mechanisms will be elucidated by several measurements (MRI, inflammation markers, lipid profile, omega-3-index). Genotyping for common learning- and metabolism-relevant polymorphisms will also be conducted.

Results: Up to now, cross-secional data of 35 MCI patients at the age of 70.4 ± 6.5 years are available. More than 80% of the patients had an omega-3-index <8% ($6.5 \pm 1.4\%$, n = 34) and therefore show a higher cardiovascular risk. At baseline, a negative association was found between the omega-3-index and interleukine 1a (r = -0.411, p<0.05). A significant increase of the omega-3-index (p=0.007) was observed in patients who already finished the trial.

Do Saturated Fats really damage your Cardiovascular Health or just your wealth (if your Danish)?

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There is something rotten in the state of Denmark! In October 2011, the Danish population have been subjected to a new tax on saturated fat of 16 Danish Kroner per kg, which has at a stroke increased the cost of butter, margarine and whipped cream by 14, 21 and 12% respectively (1). According to the Danish government-funded Forebyggelses Kommisionen (Prevention Commission), which assesses the nation's health priorities, if the variable tax is levied for 10 years it will increase average life expectancy amongst the Danish population by 5.5 days - a massive 0.15%. Even if the science behind this proven, is it fair to discriminate against tasty and functional animal fats at a fair price for such a paltry improvement?

This paper will use arguments from a debate entitled "The redemption of the British Breakfast. Re-evaluating the importance of the role of saturated fats and cholesterol in the diets of healthy adults" held at the SCI HQ in London in September 2011. Whilst convincing mechanisms exist for the role of narrow particle size oxidized LDL particles in building atherosclerotic plaques, there is little convincing evidence, in terms of population studies or clinical trials, to show that saturated fats pose any risk to the heart health of healthy adults. However, there is new evidence that swapping saturated fats for PUFA in the diets (but not carbohydrates) does reduce the risk of cardiovascular events, but is this only due to beneficial effects of omega 3 fatty acids?

The new Danish tax will disproportionately affect the poorest members of their community and yet a recent study by Tiffin and Arnout that the disease risk to the individual will not reduce significantly.

Tiffin R & Arnoult M, Eur J Clin Nutr , 2011,65:427-433.

Nutrition and Health Claims in the European Union in relation to Omega 3 Fatty Acids – existing and possible future effects on New Product Launches

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The current Nutritional Labeling Legislation in Europe for Omega 3 fatty acids has curiously skewed new product development in the food sector. The biggest category is fish products that already contained sufficient EPA/DHA to meet the contents requirements to make an omega 3 fatty acid contents claim. Where Omega 3 fatty acids have been added to new products, in the vast majority of cases this has been done by adding small quantities of ALA containing vegetable oils (e.g. rape/canola, linseed/flax), even in the case of some baby foods. Whilst manufacturers are directed by legislation to make it clear on pack the proportions of each omega 3 fatty acid present, this is usually difficult to find or not present at all. As a result the current confusion of European consumers regarding the relative benefits of DHA, EPA and ALA is becoming further engrained, and likely to lead to a further worrying reduction in EPA/DHA intake.

By the time of this conference, the process of individual European countries adopting article 13.1, 13.5 and 14 Health Claims into their respective legislation will be well underway. New product development came to a near halt whilst the EU European Food Safety Authorities Nutrition Dietetic and Allergies panel produced its opinion. In relation to Omega 3 fatty acids, some of these opinions are already outdated scientifically, and again, may mislead the European public as they fail to recognize the minimal, and nutritionally ineffective, conversion level of ALA to DHA in most of the human population. On the positive side, specific claims for health benefits of DHA and EPA will be allowed for the first time. This paper will address the likely effects on NPD in 2013 and beyond.

Effect of omega-3 rich eggs and fish on the proportion of highly unsaturated fatty acids in red blood cell membrane phosphatidylcholine and phosphatidylethanolamine of human subjects

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Objective: To determine the effect of n-3 fatty acids from fish and eggs on the proportion of highly unsaturated fatty acids (HUFA) in red blood cell membrane phosphatidylcholine (RCM PC) and phosphatidylethanolamine (RCM PE) of human subjects.

Methodology: After a 4-weeks baseline-diet, 46 women and 21 men were randomly allocated for 8-weeks to a habitual-diet and either Diet-1 (control - seven regular eggs/week); or Diet-2 (seven alpha-linolenic acid (ALA) rich eggs/week); or Diet-3 (three portions of fish/week). Fasting blood samples were analysed at the start and at the end of the intervention period for the fatty acid composition of RCM PC and PE, and the proportions of n-3 and n-6 HUFA (n-3: C20:5+C22:5+C22:6; n-6: C20:2+C20:3+C20:4+C22:4+C22-5) were calculated. Treatment effects were estimated using Analysis of Covariance with the baseline value as covariate.

Results: At baseline the proportions of n-3 HUFA in RCM PC on Diets 1-3 were 27%, 25% and 27% and after intervention 24%, 26% and 31%, respectively. The proportion of n-3 HUFA in RCM PC was higher on Diet-2 than on Diet-1 (treatment effect [TE] 3%; 95% confidence intervals [CI]: 0.3-5%), but lower than on Diet-3 (TE 4%; CI: 2-7%). The proportion of n-6 HUFA in RCM PC was lower on Diet-2 than on Diet-1 (TE 3%; CI: 0.3-5%), but higher than on Diet-3 (TE 4%; CI: 2-7%). The proportions of n-3 HUFA in RCM PE did not differ between Diet-1 and Diet-2, but it was higher on Diet-3 than on Diets-1 and -2 (TE 4%; CI: 2-6% and TE 3%; CI: 1-5%) respectively. The same but opposite pattern was observed for n-6 HUFA.

Conclusion: Three portions of fish per week and one egg (high in ALA) per day, can improve the proportion of n-3 HUFA in the RCM PC, but for the RCM PE only fish had a positive effect.

Differences in sebum fatty acid composition between body regions of young Japanese women

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Background - The sebum is biosynthesized in sebaceous glands and secreted from them to skin surface. Sebum excretion plays an important role in the development of acne vulgaris. Objective - The first aim of this study is to compare sebum fatty acid composition in the difference part of the body. The second aim is to investigate the relationship between sebum content and fatty acid composition of face. Design - Forty-two healthy Japanese women aged 20-22 years were recruited in Kagawa Nutrition University. The sebum was collected from face (forehead and nose), neck, chest, and back by using absorbent cotton. Especially, the collection on face was carried out under the condition of no or specified treatment. After lipid extraction from the cotton by chloroform-methanol (2:1), saponification, and fatty acid derivatization were performed, the methylated fatty acids were analyzed by using gas chromatography. Moreover, the sebum content of the right cheek was measured using a sebumeter. Results - The percentages of C14:0, C15:0, C16:0, C17:0, C18:0, and their total fatty acids (SFAs) were significantly lower in the face than other regions, respectively ($p<0.05$). On the other hand, the C16:1n-9, C18:1n-9, and their total percentages (MUFAs) were significantly higher in the face ($p<0.05$). However, there were no marked differences in the fatty acid composition between neck, chest, and back. The percentage of C18:1n-9 was significantly higher in the higher group than in lower group of sebum content. Face lotion and cream used in specified treatment contained 13% of C18:0 . The other fatty acids were not detected in the cosmetics. Conclusions - Our results suggest that there are clearly some differences in sebum fatty acid composition between regions of skin in young Japanese women, and that C18:1n-9 percentage may be related to acne occurrence in face.

Characterization and Structural Studies of Selected Seed Oils from East Africa Using ESI-FTICR-MS, NMR and GC-MS

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In the search for new sources of seed oils six local seed oils, obtained by solvent extraction (n-hexane:2-propanol, 3:1), with average yield of about 40%, from East Africa have been investigated. GC-MS analysis showed that total unsaturation in five of the seed oils ranged from 82.81 to 93.47%. Two of the seed oils: perekek, Podocarpus gracilior, (39.41%, oleic acid) and macadamia, Macadamia integrifolia, (52.21%), were oleic acid dominated, whilst linoleic acid dominated four seed oils: masineitet, Croton megalocarpus, (77.20%), kimolwet, Canthium lactescens, (72.09%), tuyuno, Balanites aegyptiaca, (39.79%) and kabaka anjagala, Aleurites moluccana, (39.82%). M. integrifolia   and A. moluccana seed oils contained unusually high amounts of palmitoleic (21.78%) and linolenic (27.42%) FAs, respectively. ESI-FTICR-MS analysis of TAG compositions revealed that P. gracilior had 11 major TAG classes dominated by C54:5 (16.50%), C56:6 (14.64%), and C56:5 (12.93%); C. megalocarpus had 6 major TAG classes predominated by C54:6 (48.02%), followed by C54:5 (20.55%) and C54:4 (10.12%); C. lactescens had 5 major TAG classes with C54:6 (37.83%) and C54:5 (23.35%) as the major classes; A. moluccana had 9 major TAG classes dominated by C54:7 (24.25%),and C54:6 (23.98%); M. integrifolia had 11 major TAG classes of which C52:3 (27.17%)and C54:3 (21.34%) were dominant; B. aegyptiaca had 5 major TAG classes with C52:3 (12.84%) and C52:4 (12.18%) being the major TAG classes. 13Carbon NMR analysis showed that the TAGs sn-2 position was exclusively was occupied by unsaturated acyl chains whereas the sn-1/3 positions were occupied by both unsaturated and saturated acyl chains. All six seed oils are good sources of unsaturated FAs for healthy dietary consumption.

The characteristics of HDL in late preterm infants

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Background: Some of preterm infants are known to develop neurological disability in school age even if they had no perinatal complication. It has been speculated that apolipoprotein E (apoE)-rich HDL is the key cholesterol carrier to the central nervous system in the fetus. In this study, we investigated about the

characteristics of HDL and apoE levels in late preterm infants (LPIs) whose gestation of 34-37th weeks and compared them with those of term infants (TIs).

Subjects and methods: Eighty-one neonates (25 LPIs, 56 TIs) who were born vaginally or by cesarian section at 34-41th week of gestation in maternity ward of Nihon University during 2 years between 2007-2009. Their birth weights were appropriate for gestational age. We collected blood from them at birth and at 1 month of age. Cholesterol and triglyceride levels in each lipoprotein subclass were measured by HPLC with gel permeation columns. ApoE level was also determined by turbidimetric immunoassay. Feeding information was obtained from each mother 1 month after each child's birth.

Results: The distribution of feeding source was not different between TIs and LPIs at 1 month of age. In TIs, there were positive correlations between very large, large subclasses of HDL and apoE levels at birth (r=0.48, p=0.0017; r=0.41, p=0.0072, respectively), but not in LPIs. In the relationship between gestational age and apoE, there were negative associations in both samples of at birth and at 1 month (r=-0.52, p<0.0001; r=-0.67, p<0.0001, respectively). After birth, tracking phenomenon with respect to apoE was observed only in TIs.

Discussion: Fetal HDL is thought to be an acceptor of apoE secreted from placenta. In this study, LPI's HDL was showed to have no correlation with apoE and this seemed to be one of the major causes of future developmental problems in LPIs.

Milk fatty acids as determined by maternal diet alter gene expression and metabolic profiles in developing liver

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The long chain (LC) n-3 fatty acids regulate metabolism in adult liver, promoting increased fat oxidation and decreased fat synthesis and glucose oxidation. We recently showed that the perinatal supply of n-3 fatty acids is relevant to metabolic regulation in the neonate, possibly facilitating the transition from fetal to infant nutrition. The suckling animal is also of interest as the milk provides greater than 50% energy from fat, although the milk-fed infant does not develop liver steatosis or other complications associated with high fat diets. Our aim was to determine whether the n-3 fatty acid supply in milk is relevant to metabolic regulation in the infant rat. Rats were fed diets designed to alter the n-3 and n-6 fatty acid content of milk. The diets were prepared with high 18:1 safflower oil, high 18:2n-6 safflower oil, or a blend of high 18:1 safflower and fish oils, providing LC n-3 fatty acids. Blood and livers were collected at 15 days of age. The composition of the maternal diets was reflected in the fatty acid composition of milk and infant liver. Higher 20:5n-3 and 22:6n-3 in milk led to lower hepatic mRNA for enzymes of fatty acid oxidation and synthesis: Cpt1a, Fasn, Acaca, Slc1a25, Acly, Elov5, Fads2, Scd1; ketogenesis: Hmgcs2; glycolysis: Pklr; and the enzyme that converts serine to glycine: Shmt1. Higher milk 18:2n-6 led to a similar but weaker inhibition of Fasn, Acaca, Slc1a25, Acly Elov5, Fads2 and Pklr compared to high 18:1 milk. Higher milk 20:5n-3 and 22:6n-3 also led to lower liver glycine and higher methionine, with no effect on plasma triglycerides, cholesterol, ketones and insulin. Our results suggest that milk n-3 fatty acids influence gene expression across fatty acid, glucose and amino acid pathways in the infant liver, for which the physiological implications are unknown.

Aging does not affect plasma DHA levels or response to supplementation

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It has been suggested that aging individuals have higher plasma levels of DHA omega-3 fatty acids compared to younger adults and that responsiveness to supplementation may be altered (de Groot, 2009, Vandal, 2008). Potential mechanisms for these observations include altered fatty acid metabolism or changes in dietary patterns. A cross-study analysis of 265 healthy adults across 4 supplementation studies, ranging in age from 18-92 years, demonstrates no significant effect of age on baseline plasma phospholipid DHA levels (overall mean= 3.19± 0.95 wt%; p=0.098). More importantly, supplementation with 900-1000mg/d DHA for >1month more than doubled plasma phospholipid DHA levels (overall mean=6.53± 1.74 wt%, p<0.001) and plasma fatty acid response was not affected by age (Tukey test,

p>0.05). No gender-specific differences were seen in baseline or post-supplement plasma phospholipid DHA levels. DHA dietary intake (measured by FFQ) was on average about 100 mg/d and was not significantly altered over the course of supplementation (p=0.31). The DHA doses administered approach saturation in plasma (Arterburn, 2006) and 900 mg/d DHA significantly improves memory and learning performance in healthy older adults with age-related cognitive decline (Yurko-Mauro, 2010). The robust DHA plasma response to supplementation in the elderly is corroborated by similar increases in plasma and CSF phospholipid DHA in Alzheimer's disease patients (ages: 50-95) who were administered 2 g/d DHA over 18 months (Quinn, 2010). This finding suggests that DHA is available to brain tissue in older adults. Collectively, the data indicate that aging does not affect plasma DHA levels or response to supplementation.

Comparison of maternal and umbilical plasma and erythrocyte n-3 and n-6 fatty acid status in river/lake, coastal and inland regions of China

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There is limited information regarding long chain PUFA(LCPUFA) status in Chinese pregnant women. The aim of this cross sectional study was to investigate n-3 and n-6 LCPUFA status in pregnant women from different regions of China. Fatty acid profiles of blood samples and their association with diet were analyzed. Median intakes (mg) of arachidonic acid (AA), EPA and DHA were 101.1, 27.9 and 41.8 in river/lake group (n=41), 140.1, 64.6 and 93.9 in coastal group (n=42), and 170.2, 12.1 and 41.1 in inland group (n=40). Mean dietary n-6 to n-3 PUFA ratio was 5.8, 17.8 and 12.2 in river/lake, coastal and inland groups, respectively. AA level (%) of maternal erythrocyte phosphatidylcholine(PC) in inland group (7.0+1.9) was significantly higher than that of coastal and river/lake groups (6.2+2.0 and 5.9+3.0) respectively. DHA level (%) in maternal and umbilical plasma PC were 4.5+1.6 and 6.0(4.0-10.6) in river/lake group and 5.1+2.0 and 5.7(4.1-11.1) in coastal group, both significantly higher than that in inland group (3.6+1.8 and 4.0(3.1-10.1)). DHA level (%) of maternal erythrocyte PC was comparable between river/lake and inland groups (6.3+3.0 and 6.0+2.5) but both significantly lower than that in coastal group (10.5+5.4). DHA level in umbilical erythrocyte PC was comparable among the three groups. There was a positive association between EPA&DHA intake and the level of these fatty acids in maternal erythrocytes. In conclusion, dietary n-6 to n-3 PUFAs ratio is higher but DHA&EPA intake is lower in Chinese pregnant women and this is reflected in maternal and umbilical plasma and erythrocytes.

Suboptimal docosahexaenoic acid intrauterine accretion in gestational diabetic pregnancies may mediate the adverse metabolic impact on fetal insulin sensitivity

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Background: International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends ≥300 mg/day intake of docosahexaenoic acid (DHA) for pregnant women. Low blood DHA level is associated with insulin resistance in adulthood. Fetal insulin sensitivity is impaired in gestational diabetic pregnancies. It is unknown whether suboptimal intake or accretion of DHA is involved in impaired fetal insulin sensitivity in gestational diabetic pregnancies.

Objective: To determine whether maternal intake or fetal accretion of DHA is associated with fetal insulin sensitivity in gestational diabetic pregnancies.

Methods: A singleton pregnancy cohort study in Montreal, Canada (n=307). A food frequency questionnaire was completed at 24-28 weeks of gestation. Maternal and cord plasma fatty acids were measured in a subset of mother-newborn pairs (n=132).

Results: The median intake of DHA was ~100 mg/day, and not significantly different between gestational diabetic (n=27) and non-diabetic (n=280) women (P=0.64). Over 90% women had DHA intake below 300 mg/day. Maternal (24-28 and 32-35 weeks) and cord plasma DHA levels were highly correlated (r≥0.44, P<0.0001). Maternal plasma DHA levels at 24-28 and 32-35 weeks were not significantly different between gestational diabetic and non-diabetic women (P>0.8), but cord plasma DHA levels were significantly lower in gestational diabetic (n=12) vs. non-diabetic pregnancies (n=120) (mean: 2.7 vs. 3.3%, P=0.045). Higher maternal insulin resistance (plasma proinsulin level) at 24-28 weeks was associated with lower cord plasma DHA (r=-0.18, P=0.037) levels. Lower DHA levels in maternal plasma at 32-35 weeks (r=-0.20, P=0.02) or cord plasma (r=-0.34, P<0.0001) were associated with higher fetal insulin resistance (cord plasma proinsulin level).

Conclusions: Our data suggest impaired fetal accretion of DHA in gestational diabetic pregnancies, which may partly mediate the deleterious effect on fetal insulin sensitivity. High intake of DHA may be warranted in gestational diabetic pregnancies to prevent the adverse metabolic impact on fetuses.