ORAL ABSTRACTS BY SCHEDULE

The following abstracts are for all Oral Presentations and organized by their place on the Congress Program schedule. We have provided a reference number after the Abstract Title so that you may find the corresponding day and time.

Reference number is ordered:
day.session.time.order > xx.xx.xxxx.xxx
Example below: 01.01.1040.001,
01=Sunday, 01=Session #1, 1040 = 1040 AM Start, 001 = First presentation of the Session

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Novel Pro-Resolving Mediators in Inflammation: Resolvins, Protectins and Maresins 01.01.1040.001

Presenter Last Name: **Serhan**

Novel cellular mechanisms and mediators involved in the resolution of self–limited inflammation gave new n-3 pathways in host defense, tissue injury and nutrition. Using a systems approach coupled with lipid mediator lipidomics and resolving inflammatory exudates, we uncovered several new families of potent bioactive mediators derived from n-3 essential fatty acids precursors EPA, DHA and DPA. This presentation updates our advances on the biosynthesis and functions of this novel genus of specialized pro-resolving mediators (SPM). SPM include 3 families of mediators: resolvins, protectins, maresins and the most recent addition, their n-3 DPA-derived forms all function as immunoresolvents (Dalli et. al. 2013). These n-3 autacoids possess potent anti-inflammatory, pro-resolving and microbial clearance actions in animal models. Each SPM proved potent, cell type-specific and stereoselective with human cells and in animal diseases models. Endogenous formation of resolvins and protectins and their

organ-protective roles were confirmed, extended into human clinical trials and identified in human tissues that will be presented. SPM display potent actions in, mouse models of infection, obesity colitis, arthritis, as well as reduce pain (Spite et. al. Cell Metabolism 2013), Resolvins regulate specific microRNAs during resolution via GPC receptor-dependent mechanisms. Identification of SPM biosynthesized locally and temporally during acute inflammatory responses demonstrated that resolution of acute inflammation is an active programmed process that also stimulates tissue regeneration. These findings change the >200 year old concept that resolution is a passive process. Collectively, they indicate that failed resolution pathways may underlie many prevalent diseases associated with uncontrolled inflammation and open potential for resolution-based pharmacology.

Author acknowledges NIH grants GM095467 and NS067686

The Good, the Bad and the Ugly: Diversity of airway actions of eicosanoids may be used to improve the treatment of asthma

01.01.1040.002

Presenter Last Name: **Dahlen**

The presentation will review recent findings in asthmatics and human tissues to illustrate how different arachidonic acid-derived lipid mediators (eicosanoids) have distinct roles in the pathobiology of asthma. Whereas prostaglandin (PG) E2 mediates inflammation and pain in most tissues, it has a bronchoprotective and anti-inflammatory action in the lung. Aggressive attacks of asthma are thus precipitated in subjects with the syndrome of aspirin/NSAID-intolerant asthma when the protective effect of PGE2 on EP2 receptors on airway mast cells is lost by inhibition of its local biosynthesis catalysed by COX-1 in airways. In contrast, inhibition of COX-2 is tolerated by asthmatics.

Prostaglandin D2 is the major eicosanoid released by activated mast cells. It produces bronchoconstriction by activation of TP receptors in the airways and may contribute to inflammation by stimulation of chemotactic responses via DP2 (CRTH2) receptors. Already at baseline, subjects with asthma have elevated levels of PGD2 as indicated by increased urinary excretion of its two main urinary metabolites tetranorPGDM and 2,3-dinor-11-β-PGF2α. When an asthma attack is provoked by allergen exposure or exercise, there is a further increase in the urinary excretion of PGD2 metabolites.

The cysteinyl-leukotrienes (CysLTs: LTC4, LTD4 and LTE4) are however the most potent bronchoconstrictors within the eicosanoids system. The constriction as well as pro-inflammatory effects are in humans caused solely by activation of CysLT1 receptors. In a recent study of biomarkers in asthma, it was found that

increased urinary excretion of LTE4 was the strongest predictor of severe asthma and depressed lung function. Moreover, urinary LTE4 levels correlated with high total IgE, exhaled NO and eosinophils in sputum or blood, suggesting that urinary LTE4 is a sensitive biomarker of Th2-driven airway inflammation.

It is concluded that combined antagonism of PGD2 and CysLTs has potential as new asthma therapy as well as inhaled EP2 agonists.

Inhibitors against Microsomal prostaglandin E synthase-1 – where do we stand? 01.01.1040.003

Presenter Last Name: **Jacobsson**

Microsomal prostaglandin E synthase-1 (mPGES-1) constitutes a drug target for inflammation and cancer. mPGES-1 catalyzes the biosynthesis of prostaglandin (PG) E2 from cyclooxygenase (Cox) -derived PGH2, which in turn is derived from arachidonic acid. mPGES-1 is mainly associated with inflammation and it is known to be up regulated by various pro-inflammatory cytokines like IL-1 beta and TNF-alpha. Mice devoid of mPGES-1 activity display resistance to development of experimental arthritis, fever, pain, symptoms following a stroke, atherosclerosis and breathing anomalies induced by hypoxia. Conversely, the enzyme seems to have a protective role in wound healing and remodeling after after myocardial infarction. Inhibitors of mPGES-1 have been developed by several groups. However, their characterization in animal models of inflammation, or other models previously used to study mPGES-1 knock-out mice, remains limited. One reason is the fact that a majority of the potent inhibitors of human mPGES-1 are significantly less potent or even completely inactive towards rodent mPGES-1. In addition, the impact of mPGES-1 inhibitors on the prostaglandin profile should be further investigated in various cells and in vivo systems, as their effects but also side-effects will largely depend on the particular prostaglandin profile they elicit. In this presentation, an update will be provided on the recent characterization of mPGES-1 inhibitors.

This project is supported by funds from the Swedish Research Council

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15-lipoxygenase: a novel drug target for treatment of respiratory inflammatory diseases 01.01.1040.004

Presenter Last Name: **Claesson**

Human 15-lipoxygenase-1 (15-LO-1) converts arachidonic acid to the pro-inflammatory 15-HETE, eoxins and other putative biologically active compounds. Human airway epithelial cells, eosinophils and subsets of mast cells and dendritic cells constitutively express high amounts of 15-LO-1. In particular eosinophils but also mast cells and airway epithelial cells can produce eoxins. Many reports have demonstrated a significantly increased expression and activity of 15-LO-1 in the lung in asthmatics in comparison with healthy subjects.

Mice deficient of 12/15-LO, the ortholog to human 15-LO-1, had an attenuated allergic airway inflammation and decreased mucus secretion compared to wild type controls. The function of 15-lipoxygenase-1 in dendritic cells will also be discussed.

Omega-3 fatty acid-derived neurodevelopment and neuroprotective function 01.02.1040.001

Presenter Last Name: **Kim**

Docosahexaenoic acid (DHA, 22:6n-3) is particularly enriched in neuronal tissues mainly as membrane phospholipids. Maintenance of a high DHA concentration in brain is essential for proper neurodevelopment and function, suggesting an important neurotrophic role played by this fatty acid. DHA promotes neuronal survival primarily due to its unique property to increase phosphatidylserine (PS), the major anionic phospholipid in neuronal membranes. Membrane translocation and activation of key kinases such as Raf, PKC and Akt is PS-dependent, providing a target for DHA-mediated neuroprotection. DHA is also metabolized to a potent neurogenic and synaptogenic compound synaptamide (N-docosahexaenoylethanoamide), inducing neuronal differentiation of neural stem cells and promoting neurite growth, synaptogenesis and glutamatergic synaptic function in developing neurons. The DHA status in the brain has significant impact on the development of neurons as well as recovery outcome after brain injury. Molecular and signaling mechanisms underlying DHA-mediated neurotrophic and neuroprotective effects will be discussed along with potential targets for drug development.

Coordinated transcriptional regulation of arachidonic and docosahexaenoic acid cascade enzymes during human brain development and aging 01.02.1040.002

Presenter Last Name: **Rapoport**

Background. In mammalian brain, arachidonic and docosahexaenoic acids (AA and DHA) participate in membrane synthesis and neurotransmission. They are found in sn-2 positions of membrane phospholipids, and are metabolized via coupled enzymatic reactions within specific metabolic cascades, after being released by a phospholipase A2 (PLA2). The extent to which these coupled metabolic cascade reactions are regulated transcriptionally is unclear. Hypothesis. Changes in cooperative transcription of genes for enzymes within the AA and DHA cascades underlie specific functional and structural changes during brain development and aging. Methods. A database (BrainCloud) from non-pathological postmortem human prefrontal cortex was probed to quantify age changes in mRNA levels of 34 AA and DHA metabolizing genes. BrainCloud provides mRNA expression levels of 30,176 gene expression probes in postmortem brain from 231 non-neuropathological subjects aged 0 to 78 years. Results. mRNA expression patterns could be divided into significantly distinct Development (0 to 20 years) and Aging (21 to 78 years) periods . Expression of genes for cPLA2 (PLA2G4A, PLA2G4B, PLA2G4C), cyclooxygenase-1 (PTGS1) and -2 (PTGS2), acyl-CoA transferase-4 (ACSL4) and other selective AA cascade enzymes correlated closely with age and with each other during Development, less so during Aging. Expression of DHA cascade genes (PLA2G6, LPCAT4) was less inter-correlated in each period; DHA expression genes often changed in the opposite direction to expression of AA cascade genes. Except for PLA2G4A and PTGS2 at 1q25, whose expression declined together during Aging, highly inter-correlated genes were at distant chromosomal loci. Conclusions. Changes in coordinated age-related gene transcription during human brain aging regulate changes in enzyme coupling and metabolism within the AA and DHA cascades. These latter changes in turn underlie phenotypic age changes in synaptic growth and pruning, neurotransmission, myelination and other brain processes. Healthy brain aging does not show upregulated PLA2G4 and PTGS2 expression as in Alzheimer disease.

Providing male rats deficient in iron and n-3 fatty acids with iron and alpha-linolenic acid alone affects brain serotonin and cognition differently from combined provision 01.02.1040.003

Presenter Last Name: **Baumgartner**

Background: We recently showed that a combined deficiency of iron (ID) and n-3 fatty acids (n-3 FAD) in rats disrupts brain monoamine metabolism and produces greater memory deficits than ID or n-3 FAD alone. Providing these double-deficient rats with either iron (Fe) or preformed docosahexaenoic acid (DHA)/eicosapentaenoic acid (EPA) alone affected brain monoamine pathways differently from combined repletion and even exacerbated cognitive deficits associated with double-deficiency. Iron is a co-factor of the enzymes responsible for the conversion of alpha-linolenic acid (ALA) to EPA and DHA, thus, the

provision of ALA with Fe might be more effective in restoring brain EPA and DHA and improving cognition in double-deficient rats than ALA alone. Objective: We examined whether providing double-deficient rats with ALA and Fe, alone or in combination, can correct deficits in monoamine metabolism and cognition associated with double-deficiency. Procedure: Using a 2x2 design, male rats with concurrent ID and n-3 FAD were fed an Fe+ALA, Fe+n-3 FAD, ID+ALA, or ID+n-3 FAD diet for 5 weeks postweaning. Biochemical measures, and spatial working and reference memory (using the Morris water maze) were compared to age-matched controls. Results: In the hippocampus, we found a significant FexALA interaction on DHA: compared to the group receiving ALA alone, DHA was significantly higher (+26%) in the Fe+ALA group. In the olfactory bulb and frontal cortex, we found significant antagonistic FexALA interactions on serotonin concentrations. Provision of ALA alone impaired working memory compared with controls. In the reference memory task ALA provided in combination with Fe significantly improved performance. Conclusions: These results indicate that providing ALA alone to double-deficient rats may have detrimental effects on spatial memory. However, when provided in combination with Fe, ALA might be beneficial. This may be explained by the enhancing effect of Fe on the conversion of ALA to DHA in the hippocampus.

DOCOSAHEXAENOIC ACID AND BRAIN PATHOLOGY

01.02.1040.004

Presenter Last Name: **Michael-Titus**

There is emerging evidence across a range of disease models in experimental neurology that docosahexaenoic acid has therapeutic and prophylactic potential. Its efficacy has been suggested in both acute injury and neurodegeneration in the central nervous system. The endpoints used to characterize the profile of this fatty acid range from behavioural functional outcomes to histological evidence of tissue protection and increased cell survival. Furthermore, supporting evidence continues to be generated through use of transgenic mice with altered production of long chain omega-3 fatty acids, or through the use of dietary-induced variations in tissue levels. The experimental evidence suggests that administration of docosahexaenoic acid has an impact from both a neuroprotection and a neuroregeneration perspective. Several key questions remain to be addressed from a drug development point of view in order to make the use of docosahexaenoic acid preparations clinically translatable in patients with acute or chronic brain pathology. Some of these questions, pertaining to both pharmacokinetic and pharmacodynamic aspects, will be addressed, with specific examples.

Maintaining brain polyunsaturated fatty acid concentrations:

Uptake and rapid metabolism. 01.02.1040.005

Presenter Last Name: **Bazinet**

The brain had a unique polyunsaturated fatty acid (PUFA) composition, being enriched with both arachidonic acid and docosahexaenoic acid while being very low in other PUFA such as eicosapentaenoic acid (EPA). In this paper, I will first review candidate mechanisms of fatty acid uptake into the brain, including lipoproteins, lysophospholipids and unesterified fatty acids from rodent models. I will then discuss recent kinetic approaches that support the hypothesis the plasma unesterified fatty acids rapidly enter the brain and will compare their rate of uptake into the brain to independently calculated rates of loss. While it is possible that fatty acids enter the brain via active transport, this does not appear to be necessary for fatty acid uptake into the brain and brain uptake of fatty acids cannot be outcompeted. Consistent with a non-selective uptake of fatty acids, EPA, despite its very low brain levels, enters the brain at a similar rate as other fatty acids. Upon EPA's entry into the brain, it is rapidly and extensively beta-oxidized, converted to longer chain PUFA and not recycled within brain phospholipids. The rate by which fatty acids enter the brain can be compared to dietary intakes and synthesis rates. Furthermore, identifying the pools that are bioavailable to the brain could lead to novel approaches to target the brain with PUFA and image fatty acid metabolism in health and disease.

Maternal and infant nutrition 01.03.1040.001

Presenter Last Name: **Makrides**

Docosahexaenoic acid (DHA) has many postulated roles during the perinatal periodincluding extending the period of gestation, increasing birth weight, enhancing neurodevelopment and reducing the risk of allergic disease in early postnatal life. However, it has been difficult to elucidate the extent of benefit for general populations as different studies have been specifically designed to assess particular outcomes. For example, many studies designed to assess the effect of DHA supplementation during pregnancy on childhood neurodevelopmental outcomes have excluded children born prematurely and do not contain data relating to the duration of pregnancy. Because of its large sample size and relatively broad inclusion criteria, the DOMInO (DHA to Optimise Mother Infant Outcome) trial offers the opportunity to explore the effect of prenatal DHA supplementation of multiple outcomes as well as exploring whether different population subgroups respond differently to DHA supplementation. Overall, the DOMInO trial has reported that prenatal DHA supplementation increases the duration of gestation (reduces early preterm birth and increases post-term birth), increases birth weight (reduces birth weight <2500g and increased birth weight >4000g), has no clinically meaningful effect on early

childhood neurodevelopment but reduces the risk of atopic eczema and sensitisation in the first year of life. As some of these outcomes may be perceived to be both positive and negative for the general population, it is important to understand whether there are differential responses in different population sub-groups. Women who were non-smokers are study entry were more likely than smokers to respond to parental DHA supplementation. DHA supplemented non-smokers experienced a lower risk of preterm birth before 34 weeks (RR 0.33, 95% CI 0.13 to 0.83, $p<0.05$) while the groups did not differ in women who smoked (RR 0.89, 95% CI 0.34 to 2.38). As expected the birth weight of infants from women who smoked was lower than that of women who did not, but prenatal DHA supplementation in non-smoking women further increased birth weight, reduced the risk of birth weight >2500g, increased the risk of birth weight >4000g and large for gestational age, while there was no effect of DHA supplementation in women who smoked. These data suggest that the increase in birth weight in non-smokers is associated both increased duration of gestation and increased fetal growth. The presentation will also explore the responsiveness of prenatal DHA supplementation according to maternal education.

LCPUFA supplementation in infancy improves response inhibition in childhood 01.03.1040.002

Presenter Last Name: **Gustafson**

We investigated the effect of LCPUFA on response inhibition in a follow-up study of 54 term infants randomized to receive formula with a constant level of ARA (0.64% TFA) and different levels of DHA (0.32, 0.64, 0.96% TFA) or no LCPUFA from birth to 12 months. At mean age 5.5 ±0.03 years, children participated in a Go/No-Go task. Children were instructed to press a button when a cartoon image of a fish appeared (Go, 65% of trials) or inhibit the response when sharks appeared (No-Go, 35%). Event-related potentials (ERP) were measured via 33 scalp electrodes. Inhibition-related ERP components, N2 (300~500 ms) and P3 (500~700 ms) were identified. Supplemented groups were collapsed. Peak amplitudes of the grand-averaged ERP were subjected to a mixed-design ANOVA calculated on diet group (LCPUFA vs. no LCPUFA) by condition (Go vs. No-Go) as a within-subject factor. For both N2 ($F(1,52) = 15.23$, $p<0.001$) and P3 ($F(1,52) = 7.67$, $p=0.008$) components, statistically significant two-way interactions were observed, attributable to significantly greater component amplitudes in the supplemented group for the No-Go condition (N2, $p=0.034$; P3, $p = 0.012$). Neither component was influenced by LCPUFA for the Go condition. The condition effect that greater No-Go than Go was found in N2 ($p<0.001$) and P3 ($p=0.053$) was only present in supplemented group ($p<0.001$). Topographical ANOVA comparing the No-Go condition between groups revealed a novel microstate in the supplemented group. A negative correlation ($p=0.0095$, r =-

0.3957) between the P3 component and accuracy of the No-Go condition was found only in the supplemented group. Supplemented children showed significantly different brain responses on an executive-function task requiring rule learning and inhibitory control by responding more effectively and less impulsively, suggesting a positive effect of LCPUFA in neurocognitive function. Supported by an Independent Investigator Trial grant from Mead Johnson Nutrition: Clinical Trial NCT00753818

Four Year Follow-up of Children Born to Women in a Randomized Controlled Trial of DHA Supplementation during Pregnancy 01.03.1040.003

Presenter Last Name: **Gould**

Background: We investigated the effect of DHA supplementation during pregnancy on children's neurodevelopment at four-years of age to resolve controversies regarding long-term benefits of supplementation despite common use during pregnancy. Method: We conducted a follow-up of children born to mothers enrolled in the DOMInO (DHA to Optimize Mother Infant Outcome) RCT, conducted between June 2010 and September 2012. Pregnant women were supplemented with DHA-rich fish oil capsules (providing 800 mg DHA/d) or vegetable oil capsules (control group) from 21 weeks gestation until birth. The primary outcome was general cognitive ability at four years of age, as measured by the General Conceptual Ability (GCA) score from the Differential Ability Scales Second Edition (DAS II). Executive functioning was assessed objectively by a psychologist as well as by validated parent questionnaires along with behaviour. Results: Of the 703 children eligible, 646 (92%) consented to the follow-up study and were included in the analysis (treatment n=313, control n=333). GCA scores of children in the DHA group did not differ from children in the control group (adjusted mean difference 0.29, 95% confidence interval -1.35 to 1.93, P=.73). Objectively assessed executive functioning with the Day-Night Stroop did not differ between the groups, despite fewer preterm infants in the treatment group. However the DHA group children had poorer scores on some but not all parent-reported indices of executive functioning (emergent meta-cognition and plan/organise) as well as behaviour (total difficulties and hyperactivity). Conclusions: Increasing prenatal DHA exposure does not improve children's neurodevelopment at four years of age.

FADS SNPs Are Associated with Behavioral Outcomes in Children in a Gender-Specific Way 01.03.1040.004

Presenter Last Name: **Lauritzen**

Background: DHA accretes in the brain during the growth spurt, but results regarding a potential programming effect on cognitive function and behavior in humans are inconclusive. DHA can be supplied by the diet or synthesized from α-linoleic acid, and the biosynthetic capacity is modified by single nucleotide polymorphisms (SNPs) in the FADS-gene cluster. Objective: To investigate if behavioral outcomes in childhood were associated with three FADS tag-SNPs previously found to have opposing and allele number-dependent effects on infant erythrocyte DHA. Minor allele carriers of rs1535 had increased DHA, whereas those with minor alleles of rs174448 and rs174575 had decreased DHA (effect size around 0.5%-point per allele). Design: At 36 months we assessed psychomotor development by the Ages & Stages Questionnaire (n»258) and physical activity by accelerometry (n=231) in children from the SKOT cohort. Blood samples were taken to determine erythrocyte DHA-status (n=192) and the three FADS tag-SNPs (n=255). All outcomes were analyzed using multiple regression models including the three SNPs, SNP-gender interactions, and adjustment for parental education, siblings, birth-weight, and duration of breastfeeding. Results: SNP-gender interactions were found for both communication and problem solving (p=0.008-0.025). Relative to wild type individuals female rs174448 heterozygotes had better communication (b=4.8[95%CI: 0.7;9.0]) and problem solving (4.7[0.6;8.8]), whereas the associations were negative in boys (-4.9[-8.9;-1.0] and -2.0[-5.7;1.7], respectively). Similar results were seen for rs174575 and communication, but all associations for rs1535 were in the opposite direction (communication: boys 6.3[0.4;12.3] and girls: -5.2[-10.7;0.2] and for problem solving most pronounced in homozygotes (boys=11.9[-0.6;24.3] and girls=-8.7[-17.1;-0.2]). Some associations were also seen for fine motor development, but none for physical activity. Conclusion: FADS SNPs seem to have an independent and gender-specific effect on behavior in children that to some extent equalize gender differences, possibly indicating a programming effect of early DHA exposure.

Maternal but not fetal FADS gene variants modify the association between maternal DHA intake in pregnancy and birth weight 01.03.1040.005

Presenter Last Name: **Thijs**

Background: Several observational studies have shown a positive association between maternal fish intake during pregnancy and child birth weight (BW), possibly due to fish n-3 LC-PUFAs. Some randomized controlled trials have confirmed the positive effect of n-3 LC-PUFAs on BW, but others have not. n-3 LC-PUFAs can be synthesized endogenously with participation of fatty acid desaturases. SNPs in FADS genes (encoding these desaturases) influence endogenous synthesis efficiency. We hypothesize that the association between

n-3 LC-PUFA intake and BW differs between FADS SNPs genotype groups. Objectives We investigated: 1) the association between maternal DHA intake in pregnancy and BW; 2) the association between maternal and fetal FADS SNP genotypes and BW; 3) the interaction between maternal DHA intake and maternal and fetal genotypes. Methods Data on BW, maternal diet, and on several covariates were available for 2622 mother-child pairs from the KOALA Birth Cohort (The Netherlands). FADS SNP rs174545 was genotyped in a subgroup of 1516 mothers and 1515 children. Associations and gene-diet interactions were examined by multivariate linear regression. Results 100 mg/day incremental DHA intake was associated with 16 g higher BW (95% CI: 8-31 g). Mothers homozygous for the minor allele had babies almost 140 g lighter than mothers homozygous for the major allele. Maternal but not fetal FADS SNP genotype modified the association between DHA intake and BW: 100 mg/day incremental DHA intake was associated with 112 g higher BW (95% CI: 11-213 g) in women homozygous for the minor allele, while no association was found in major allele carriers. Conclusion The association between maternal DHA intake in pregnancy and BW seems to exist only in women homozygous for the minor allele for at least one FADS gene variant. Dietary advice on n-3 LC-PUFA intake in pregnancy could be especially beneficial for women with this genotype.

Fluxolipidomics of essential fatty acids 01.04.1415.001

Presenter Last Name: **Lagarde**

Essential fatty acids, including the indispensable precursors linoleic (LA, 18:2w6) and alpha-linolenic (ALA, 18:3w3), follow many metabolic pathways in various cell biological compartments. Three main aspects can be considered from a functional viewpoint: (i) their esterification into cellular phospholipids and release upon cell activation through diverse phospholipases/lipases, (ii) their oxygenation into a myriad of cyclooxygenase/lipoxygenase/cytochrome P450 or non-enzymatic bioactive products, and (iii) the metabolism of those products into inactive ones or products with other activities. These direct metabolic pathways must be completed with other related ones such as the generation of endocannabinoids and their further oxygenation, prostamides being one example. All these processes occur at different rates which means that, approaching the lipid mediator-related phenotypes requires a mediator lipidomics in function of time which may be identified as fluxolipidomics of lipid mediators and metabolites. A promising approach is the use of uniformly ^{13}C-labeled precursors and measurement of as many as possible intermediates and end-products by mass spectrometry at several time-points.
The example of oxygenated metabolites derived from omega-6 (LA and arachidonic acid/ARA) and omega-3 (ALA, eicosapentaenoic acid/EPA and docosahexaenoic acid/DHA) fatty acids, especially the bioactive metabolites, will be given. Their half-lives and degradation products will be considered for a qualitative fluxolipidomics (Lagarde et al. Mol Nutr Food Res. 2013).

Targeted Lipidomics using a Novel Integrated Microfluidics-Mass Spectrometry Technology 01.04.1415.002

Presenter Last Name: **Astarita**

Lipidomics is the comprehensive analysis of hundreds of lipid species in biological samples. Lipids play prominent roles in the physiological regulation of many key biological processes such as inflammation and neurotransmission. Alterations in lipid pathways have been associated with many diseases including cardiovascular diseases, obesity, and neurodegenerative disorders. The ability to measure the wide array of lipid species in biological samples could help our understanding of their roles in health and disease. The need for a fast, comprehensive and sensitive analysis of the hundreds of lipid species challenges both the chromatographic separation and mass spectrometry. Here we used a novel microfluidics platform packed with 1.7 µm particles for fast and robust chromatographic separation. By integrating microscale LC components into a single platform design, the device avoids problems associated with capillary connections, including manual variability, leaks, and excessive dead volume. Lipidomics analyses were conducted using small volumes of standards and extracts from typical biological samples including plasma, brain, heart or liver. Mobile phases and analysis times were similar to regular LC methods using analytical-scale columns. Data was collected using MS systems (Q-Tof and triple quadrupoles) operated in both negative and positive mode in the data-independent acquisition mode. Untargeted metabolomics analyses were conducted using Q-Tof mass spectrometers with an alternate low and elevated collision energy method to acquire both precursor and product ion information in a single analytical run. Such integrated microfluidic device is suitable for lipidomics analyses with considerable advantages compared to analytical scale LC-MS analysis, with an overall reduction in solvent consumption of > 200 x. Potential applications include large-scale lipid profiling and low-abundance lipid analyses in biological materials.

A lipidomic biosignature associated with the healthful phenotype of fat-1 transgenic mice 01.04.1415.003

Presenter Last Name: **Astarita**

Omega-3 intake has been linked to health benefits and the prevention of many chronic diseases. Current dietary intervention studies using fish oils often lack of appropriate control diets, hindering a correct evaluation of the physiologic effects

deriving from high omega-3 intake. Here we used the fat-1 transgenic mouse model to evaluate the molecular phenotype of long-term omega-3 supplementation in a controlled manner. Such transgenic mouse is able to convert omega-6 to omega-3 polyunsaturated fatty acids (PUFAs), protecting it against a wide variety of diseases including chronic inflammatory diseases and cancer. Wild type (WT) and fat-1 mice were put under an identical 6-month diet containing 10% corn oil to mimic the modern Western diet, which is enriched in omega-6 and low in omega-3 fatty acids. Unbiased lipidomic analyses revealed a significant increase in EPA cholesteryl ester (31 fold) and omega-3s phosphatidylcholine (PC) species, whereas a significant reduction in DPA omega-6 cholesteryl ester and omega-6 PC species in the plasma of the fat-1 mice as compared to their WT counterparts. Levels of unesterified arachidonic acid and DPA omega-6 were decreased whereas levels of EPA, DHA and DPA omega-3 were increased in plasma of fat-1 compared to WT mice. A targeted lipidomics profiling of over 100 oxygenated PUFA species highlighted an overall increase in anti-inflammatory or pro-resolving omega-3 PUFA metabolites, whereas a decrease in the panel of inflammatory lipid mediators. In particular, we observed a significant increase in the levels of EPA-derived 17(18)-EpETE and its metabolite 17,18 DiHETE, which are formed via cytochrome P450 (CYP450) pathway. Our study identified a characteristic lipidomic biosignature that potentially underlies the healthful phenotype associated with a balanced omega-6/omega-3 ratio. Such a lipidomic biosignature might find use in monitoring the health status and the efficacy of nutrition intervention with omega-3 PUFAs in humans.

Lipidomic analysis during vertebrate embryonic development

01.04.1415.005

Presenter Last Name: **Gibert**

Metabolism has a fundamental role in biological processes including embryonic development and tissue homeostasis. However to date, little is known about the lipid metabolic networks involved during vertebrate embryogenesis. The zebrafish embryo offers the advantage of having a fast embryonic development, needing only 5 days to become a larvae. Moreover, during development the zebrafish embryo can be considered as a “closed system”, since the embryo relies on its yolk sac reservoir for growth. To understand what lipids are involved during embryogenesis, we performed full lipidomic analysis of the yolk sac and the embryo body at different crucial stages of zebrafish embryogenesis: 1 cell-stage, 24 hours post fertilization (hpf), 48 hpf, 72 hpf and 120 hpf (end of embryogenesis). In this study, we analyse 375 lipid species that can be grouped into 24 categories. Interestingly, all lipid species identified in the yolk sac were also found in the embryo body while the reciprocal is not true. Our analysis revealed that the yolk sac is a reservoir of triglyceride. Of the main lipid classes, it contained 1.8x more total lipids, 4.3x more of both TAG and PS, 4.8x more

LPC, 2.9x more PI, 1.9x cholesterol, 2.1x of CE, and 1.7x more of PC than the embryo body; in contrast the yolk sac contained only 0.34x as much PS, and 0.16x as much as the total DHC (dihexosylceramide) in the embryo body, respectively. To investigate the role of PPARγ in lipid availability during development, we analysed the lipid changes when this gene was pharmacologically blocked during zebrafish embryogenesis. Evolution of lipid content in the yolk sac and the embryo body throughout embryogenesis will be discussed. This newly developed protocol allows us to track the lipids involved during embryogenesis and monitor lipid change due to a genetic mutation or pharmacological treatment during embryonic development.

Plasma Fatty Acid Ratios Affect Blood Gene Expression Profiles Differentially 01.04.1415.004

Presenter Last Name: **Olsen**

High blood concentrations of n-6 fatty acids (FAs) relative to n-3 FAs may lead to a “physiological switch” towards permanent low-grade inflammation, potentially influencing the onset of cardiovascular and inflammatory diseases, as well as cancer. To explore the potential effects of FA ratios prior to disease onset, we measured blood gene expression profiles and plasma FA ratios (linoleic acid/alpha-linolenic acid, LA/ALA; arachidonic acid/eicosapentaenoic acid, AA/EPA; and total n-6/n-3) in a cross-section of middle-aged Norwegian women (n = 227). After arranging samples from the highest values to the lowest for all three FA ratios (LA/ALA, AA/EPA and total n-6/n-3), the highest and lowest deciles of samples were compared. Differences in gene expression profiles were assessed by single-gene and pathway-level analyses. The LA/ALA ratio had the largest impact on gene expression profiles, with 135 differentially expressed genes, followed by the total n-6/n-3 ratio (125 genes) and the AA/EPA ratio (72 genes). All FA ratios were associated with genes related to immune processes, with a tendency for increased pro-inflammatory signaling in the highest FA ratio deciles. Lipid metabolism related to peroxisome proliferator-activated receptor γ (PPARγ) signaling was modified, with possible implications for foam cell formation and development of cardiovascular diseases. We identified higher expression levels of several autophagy marker genes, mainly in the lowest LA/ALA decile. This finding may point to the regulation of autophagy as a novel aspect of FA biology which warrants further study. Lastly, all FA ratios were associated with gene sets that included targets of specific microRNAs, and gene sets containing common promoter motifs that did not match any known transcription factors. We conclude that plasma FA ratios are associated with differences in blood gene expression profiles in this free-living population, and that affected genes and pathways may influence the onset and progression of disease.

Fatty acids in psychiatry 01.05.1415.001

Presenter Last Name: **Hibbeln**

Awaiting final submittal

Polyunsaturated fatty acids levels and initial presentation of somatic symptoms induced by interferon-alpha therapy in patients with chronic hepatitis C viral infection 01.05.1415.002

Presenter Last Name: **Chang**

Background: Somatic symptoms are common in clinical practice and are similar to sickness behaviors due to inflammatory activation after cytokine administration in animals or humans. Polyunsaturated fatty acids (PUFAs) have been suggested to mediate in somatic symptoms in depression in cross-sectional and observational studies. With the patients of hepatitis C virus infection (HCV) infection receiving interferon-alpha therapy, we investigated the role of PUFAs on the development of somatic symptoms in a prospective manner. Methods: Forty-three patients with chronic HCV ongoing interferon-alpha therapy were assessed with the Mini-International Neuropsychiatric Interview (MINI) for major depressive episodes and Neurotoxicity Rating Scale (NRS) for somatic symptoms. Patients' red blood cell (RBC) samples were collected for PUFAs analyses at baseline and two weeks during interferon-alpha therapy. Results: Fifteen out of 43 participants (34%) developed interferon-alpha-induced depression. There were no differences between the depression and non-depression groups in age, sex distribution, NRS scores, and levels of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA) at baseline. However, there was a significant negative correlation between scores of somatic symptoms and levels of EPA at week 2. The initial increases on somatic symptom scores were positively correlated to AA levels at week 2. Conclusion: The initial presentation of painful and non-painful somatic symptoms were associated with the changes in PUFA levels, which further implicates that pro-inflammatory AA and its antagonist EPA might contribute to the presentation of IFN-alpha-induced somatic symptoms in HCV patients.

Omega-3 fatty acids in the prevention of interferon-alpha-induced depression: a randomized placebo-controlled trial 01.05.1415.003

Presenter Last Name: **Su**

Background. Interferon (IFN)-α therapy for chronic hepatitis C virus (HCV) infection is frequently associated with major depressive episodes (1,2). The routine prophylaxis with antidepressants might expose patients to adverse effects, hence the need for alternative preventive interventions. Omega-3 polyunsaturated fatty acids (PUFAs) are safe and effective essential nutritional compounds used for the treatment of depression (3-5). In addition, lower erythrocyte levels of omega-3 PUFA have been associated with an increased risk of IFN-induced depression (6). Methods. We conducted a 2-week, double-blind, placebo-controlled trial, comparing eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and placebo, for the prevention of IFN-induced depression. One hundred and sixty-two patients consented to participate and were randomized to the study, and all of them completed the two-week trial; 152 participants were followed throughout the 24 weeks of IFN-α treatment, and were included in the analysis. Results. Compared with placebo, the incident rates of IFN-α-induced depression were significantly lower in EPA-, but not in DHA-treated patients (rates: 10% and 28%, respectively, vs. 30% for placebo, P=0.037). Both EPA and DHA pre-treatment significantly delayed the onset of IFN-induced depression (average weeks of onset: 12.0 and 11.7, respectively, vs. 5.3 for placebo, P=0.002). EPA and DHA were both well tolerated in this population. EPA treatment increased both EPA and DHA erythrocyte levels, but DHA only increased DHA erythrocyte levels. Conclusions. EPA appears to be effective in the prevention of depression in HCV patients received IFN-α therapy. Reference: (1) Su KP. NeuroSignals 2009; (2) Lu DY & Su KP. International Journal Neuropsychopharmacology 2013; (3) Lin PY & Su KP. Molecular Psychiatry 2012; (4) Lu DY & Su KP. Neuropsychopharmacology 2010; (5) Lin PY et al. Biological Psychiatry 2010; (6) Su KP et al. Biological Psychiatry 2010

Baseline omega-3 index correlates with aggressive and attention deficit behaviours in adult prisoners 01.05.1415.004

Presenter Last Name: **Meyer**

Background: There is emerging evidence that the supplementation of omega-3, multivitamins and minerals leads to a decrease in aggressive behaviour in prison populations. A challenge of such research is achieving statistical power against effect sizes which may be affected by the baseline omega-3 index. This may differ across regions, jurisdictions and individual metabolism. There is no published data on the baseline blood omega-3 index with studies of this kind to assess the variability of baseline blood omega-3 index in conjunction with baseline aggression and attention deficit assessments. Objective: To determine if the variance of the baseline omega-3 index is correlated with

aggressive and attention deficit behaviours in a prison population. Design: 136 adult male prisoners were recruited from South Coast Correctional Centre (SCCC), NSW Australia. A 7 point categorisation was used to quantify levels of 4 weeks aggressive behaviour from individual SCCC case notes, whereby higher scores correspond to increasingly aggressive behaviour. At baseline, study participants completed an Aggression Questionnaire (AQ) and the Brown's Attention Deficit Disorder Scales (BADDS), provided a blood sample for erythrocyte fatty acid analysis using gas chromatography and the omega-3 index was calculated. Results: The baseline omega-3 index ranged from 2.3% to 10.3% with a median of 4.7%, indicating that some participants already had substantial omega-3 intake. Assessment of aggressive and attention deficit behaviour shows that there were negative correlations between baseline omega-3 index and baseline aggression categorisation scores (r=-0.21, P=0.016); total AQ score (r=-0.234, P=0.011); Anger (r=-0.222 p=0.016); Hostility AQ (r=-0.239, P=0.009); indirect aggression (r=-0.188 p=0.042); total BADDS (r=-0.263, p=0.005); Activation (r=-0.224, p=0.016); Attention (r=-0.192, p=0.043); Effort (r=-0.253, p=0.007); Affect (r=-0.330, p=0.000) and Memory (r=-0.240, p=0.010). Conclusions: There is a high variability on baseline omega-3 status of a NSW prison population, and inmates with lower omega-3 index were more aggressive and had higher ADD scores.

Lipid mediators and receptors of resolution in Alzheimer's disease – in vivo and in vitro studies 01.05.1415.005

Presenter Last Name: **Schultzberg**

Alzheimer's disease (AD) is characterized by an inflammatory process in the brain. Omega-3 fatty acids are anti-inflammatory agents that may influence cognition in AD, and previous data show that they can stimulate the uptake of amyloid b peptide (Ab42). Their derivatives are involved in the resolution of inflammation, but little is known so far regarding their regulation in the brain and their effects on neurons and glia. The objective of this study is to analyse the levels of lipid mediators and their receptors in human post mortem brain, their effects on neurons and glia in vitro. Post mortem brain tissue from AD-patients and healthy controls were analysed by ELISA and western blots. The effects of lipid mediators on neuronal cell death and microglial phenotype were analysed in cultures of differentiated human neuroblastoma cells (SH-SY5Y) and human microglia (CHME3). The levels of the pro-resolving factors, so called specialized pro-resolving mediators (SPMs), lipoxin A4 (LXA4) and maresin (MaR1) were lower in AD than in controls, whereas the levels of receptors for SPMs, LXA4 receptor/formyl peptide receptor 2, (ALX/FPR2) and ChemR23, were higher in AD. MaR1 was found to stimulate the uptake of Ab42 by microglia and downregulate their pro-inflammatory phenotype, as well as to reduce neurodegeneration induced by staurosporine. Both neurons and microglia

express the SPM receptors ALX/FPR2 and ChemR23. The studies indicate that the resolution pathway is disturbed in AD, but that stimulation of this process may have beneficial consequences for neuronal survival in AD.

Milk membrane lipid composition 01.06.1415.001

Presenter Last Name: **Lopez**

Awaiting final submittal

Alternative transcripts in the human milk fat globule proteinogenic RNA transcriptome and a novel FADS2 transcript 01.06.1415.002

Presenter Last Name: **Kothapalli**

Introduction. Human milk fat globules (MFG) carry most fat to the nursing infant, and also carry mRNA that in principal could be transcribed. Our aim was to investigate proteinogenic RNA with emphasis on PUFA synthesis in MFG. Methods. Fresh human breastmilk sample from a single anonymous donor was centrifuged and MFG collected for mRNA isolation. An Illumina HiSeq implementing RNASeq detected >15,000 transcripts. Results and Discussion. The most highly expressed transcripts were associated with protein translation and elongation, milk protein, MFG formation, fatty acid metabolic process and transport, iron ion transport, regulation of apoptosis, and cellular component size. Six transcripts accounted for the bulk of the MFG transcriptome: CSN2 (casein beta), LALBA (lactalbumin alpha), CSN3 (casein kappa), FTH1 (ferritin heavy chain 1), CSN1S1 (casein alpha s1) and CSN1S2AP (casein alpha s2-like pseudogene). The FTH1 gene is located on chromosome 11 about 100 kb upstream of the FADS gene cluster. Alternative splice sites were identified for >3500 genes in MFG. A novel FADS2 alternative transcript (FADS2AT2) with 386 amino acids was detected and sequence determined. FADS2, FADS2AT1 and 5 out of 8 known FADS3AT were found in MFG; FADS1, FADS3AT3, and FADS3AT5 are undetectable. Transcripts involved in cellular process of translation, respiratory chain and ATP synthesis were the enriched functional gene groups identified in MFG. A lipid metabolism network using Ingenuity Pathway Analysis identified UBC as the outstanding interaction partner. Conclusion. To our knowledge this is the first report to show existence of evolutionarily conserved AT in MFG. MFG is known to contain the molecular apparatus for reverse transcription. We conclude that MFG may be a unique mRNA vector for the breast fed infant.

TLR4 and CD14 co-localization in lipid rafts: impact of stimulation status and membrane fatty acid pattern 01.06.1415.003

Presenter Last Name: **Schumann**

In this study the modulating effects of polyunsaturated fatty acids (PUFA) on macrophage membrane receptor interactions in lipid rafts were determined. The focus was on the co-localization of the key LPS signaling receptors Toll-like receptor 4 (TLR4) and cluster of differentiation 14 (CD14) with the lipid raft marker ganglioside GM1 was analyzed. RAW264.7 macrophages were supplemented with 15 µM alpha-linolenic acid (LNA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid (LA) or arachidonic acid (AA) each for 72 h and subsequently stimulated with either LPS or viable Pseudomonas aeruginosa (ATCC 10145, MOI 1) for 24 h. Co-localizations of TLR4-GM1 or CD14-GM1 were analyzed using fluorescence microscopy (BZ-9000, Keyence, Germany) and quantified (ImageJ software; National Institutes of Health, Bethesda, USA). Co-localization of TLR4 or CD14 with GM1 increased due to stimulation of unsupplemented RAW264.7 with LPS or P. aeruginosa as expected. Furthermore, an impact of the membrane fatty acid pattern on receptor interactions was determined. The consequences of PUFA supplementation of the macrophages were found to depend on the activation status of the immune cells. For unstimulated macrophages PUFA enrichment resulted in an enhancement in TLR4-GM1 as well as CD14-GM1 co-localization. For stimulated macrophages, instead, we observed an impeding action of PUFA on receptor co-localization in lipid rafts. Our data indicate that protein-protein interactions in lipid rafts are not only subject to the cellular stimulation status but also to the membrane fatty acid pattern. PUFA of both the n-3 and the n-6 family abolish the stimulation-induced co-localization of TLR4 with its co-receptor CD14 thereby inhibiting LPS signaling. The observed effects might provide an explanation for the inhibitory action of PUFA on macrophage-mediated inflammatory responses.

A diet rich in DHA prevents visual and spatial memory loss in 12 months old mice carrying the human apolipoprotein E epsilon 4 allele. 01.06.1415.004

Presenter Last Name: **Chouinard-Watkins**

Introduction: Consumption of fatty fish containing DHA is associated with a reduced risk of cognitive decline in humans, but this association is not present in carriers of the apolipoprotein E epsilon 4 allele (APOE4). We recently accumulated evidence that DHA metabolism is disturbed in APOE4 carriers, but whether this is involved in cognition is yet unknown. Objective: Using a model of

mice expressing human APOE isoforms, we investigated potential genotype-by-diet interactions on cognition. Methods: Four months old APOE3 (n = 32) and APOE4 (n = 38) mice were fed one of three isocaloric diets; a high DHA diet, a high fat (HF) diet or a control diet (n = between 11 and 15/genotype/diet) for 8 months. At 12 months, animals were tested for visual and spatial memory using the object recognition (OR) test and the Barnes maze test, respectively. A recognition index (RI) was calculated in the OR test whereas primary errors and escape latency were calculated on each of the five days of the Barnes test. Results: In the OR test, APOE3 mice fed the control or the DHA diet both had a RI significantly higher than random ($p \leq 0.01$) whereas only DHA-fed APOE4 mice had a RI higher than random ($p \leq 0.001$). In the Barnes test, there was no genotype-by-diet interaction for escape latency and primary errors. However, longer escape latency were recorded in APOE4 mice fed either the control or the HF diet compared to APOE3 mice ($p \leq 0.05$), but not in APOE4 mice fed the DHA diet. Discussion: Consistent with previously published work, APOE4 mice displayed visual and spatial memory deficits compared to APOE3 mice. Importantly, these deficits were prevented by a 8-month DHA treatment, suggesting a protective effect of DHA against APOE4-induced cognitive decline. Acknowledgments: CIHR, FRQ-S, FRQ-NT, INAF, RQRV.

Blood and brain fatty acid contents in aged rats supplemented with n-3 long-chain polyunsaturated fatty acids 01.06.1415.005

Presenter Last Name: **Buaud**

Aging is associated with changes in brain n-3 long-chain polyunsaturated fatty acid (n-3 LC-PUFA) levels, with primarily DHA playing a critical role in maintaining brain functions. However, limited data have shown the effects of aging and n-3 LC-PUFA dietary supplementation during aging on both blood and brain fatty acid compositions. In the present study, we assessed the blood and brain fatty acid contents during aging (13-month-old aged rats vs 3-week-old adult rats) and after n-3 LC-PUFA dietary supplementation in aged rats. In the blood, aging is associated to an increase in plasma DHA content whereas the DHA content remains stable in red blood cell (RBC) membranes. In the brain, aging is associated to a decrease in DHA content that was both region-specific and phospholipid class-specific, affecting mainly the striatum and more precisely the phosphatidylcholine. In n-3 LC-PUFA supplemented aged rats, the DHA contents were increased both in the blood and in the brain, to reach a brain DHA level close to, or higher than that measured in adult rats. As for aging, this repletion was region-specific and phospholipid class-specific. Furthermore, we highlighted a positive relationship between the DHA levels in RBC phosphatidylethanolamine (PE) and those in the hippocampus and prefrontal cortex in n-3 LC-PUFA supplemented aged rats. Within the framework of the formulation of a preventive nutrition to delay brain aging, these results (i)

highlight the interest of n-3 LC-PUFA dietary supplementation during aging, and (ii) suggest the possibility of using the DHA content in RBC PE as a reliable biomarker of the DHA status in specific brain regions.

Reversal of CLA-Induced Nonalcoholic Fatty Liver Disease (NAFLD) and Insulin Resistance (IR) by DHA in a Mouse Model

01.07.1415.001

Presenter Last Name: **Kelley**

Both NAFLD and IR are components of metabolic syndrome. Diets containing trans fatty acids increase both NAFLD and IR. When fed concomitantly, DHA prevented CLA-induced IR and NAFLD. Aim of this study was to determine whether DHA will reverse CLA-induced IR and NAFLD and underlying mechanisms. One group of C57BL/6N female mice was fed modified AIN diet and second was fed same diet containing 0.5% t10, c12-CLA for 8 wk. Additional 3 groups were fed CLA-containing diet for 4 wk and then transferred to reversal diets (control, control + 1.5% DHA, or CLA+ 1.5% DHA) for 4 wk. Compared to control group CLA significantly increased fasting insulin and HOMA-IR. Switching from CLA to control or control +DHA diet, but not CLA+DHA diet restored insulin and HOMA-IR to those in control group. CLA significantly increased glucose AUC in response to ITT when compared to control group, which was significantly decreased by control and control + DHA diets. Liver histology revealed a large number of macro lipid droplets in CLA group, which were markedly reduced in all other groups; least number of fat droplets was observed in CLA to control + DHA group. Expression of hepatic ACOX1 and CPT1A was decreased (>50%) and those of SCD1 and ACCA increased by 800 and 600 % by CLA when compared with control group. All 3 reversal diets restored the expression of SCD1 and ACCA to the level in control group; expressions of ACOX1 and CPT1 in CLA to control + DHA diet was 350 and 600% of that in control group, respectively. Only control + DHA diet partially restored CLA depleted adipose depots. Our results suggest that CLA increased hepatic fatty acid synthesis and decreased fatty acid oxidation and DHA increased fatty acid oxidation. Responses of different tissues to these fatty acids varied.

Pentadecanoic Acid (15:0) is a Biomarker of Dairy Food Intake and is Inversely Associated with Incident Type 2 Diabetes and Its Underlying Disorders in the IRAS cohort 01.07.1415.002

Presenter Last Name: **Santaren**

Background: Growing evidence suggests that saturated and trans fatty acids in dairy contribute to the observed protective effects of dairy intake on type 2 diabetes (T2DM) risk. Objectives: To assess the relationship of dairy biomarkers pentadecanoic acid (15:0) and trans-palmitoleic acid (trans 16:1n-7) with (a) dairy intake and (b) T2DM traits in a large multi-ethnic cohort. Methods: The study utilized data from the tri-ethnic multi-centre Insulin Resistance Atherosclerosis Study (IRAS) of 659 adults. Diabetes status was determined via oral glucose tolerance test. Frequently sampled intravenous glucose tolerance tests assessed insulin sensitivity (SI) and β-cell function (Disposition Index, DI). A validated 114-item food frequency questionnaire measured baseline diet. Serum fatty acids were quantified using gas chromatography methods. Spearman correlation and multivariate linear regression evaluated associations between dairy intake and dairy biomarkers. Logistic and linear regressions for T2DM outcomes were adjusted for demographic, lifestyle, and dietary variables. Results: The median total dairy intake was 0.79 (0.37-1.25) servings/day. 15:0 was positively and significantly associated with dairy intake (β=0.002; p<0.0001) in fully adjusted models. 15:0 was also positively associated with log SI (β=0.84; p=0.03) and log DI (β=2.21; p=0.02), as well as a 27% decreased risk in incident T2DM after 5 years (OR=0.73; p=0.02) in fully adjusted models, with significant associations in females, normoglycemics, and Hispanics, although interactions terms were not significant. Conversely, trans 16:1n-7 was not significantly correlated with either 15:0 or dairy intake, but was positively and significantly associated with partially hydrogenated fat intake (β=0.003; p<0.0001), and negatively associated with dairy intake (β=-0.001; p=0.01) in fully adjusted models. Trans 16:1n-7 was not associated with T2DM outcomes in fully adjusted models. Conclusion: The results support the hypothesis that dairy fatty acids may be associated with a reduced T2DM risk, and that 15:0 is a valid biomarker for dairy intake.

Regulation of energy homeostasis and glycerolipid metabolism in the phospholipid gene deficient mouse ETKO 01.07.1415.003

Presenter Last Name: **Bakovic**

Phosphatidylethanolamine (PE) is an important inner membrane phospholipid synthesized de novo by the CDP-ethanolamine-Kennedy pathway and by the decarboxylation of phosphatidylserine. CTP: phosphoethanolamine cytidylyltransferase (Pcyt2) is the main regulatory enzyme in the CDP-ethanolamine pathway and catalyzes the formation of CDP-ethanolamine from phosphoethanolamine. Complete deletion of the mouse Pcyt2 gene is embryonic lethal, and the single allele deficiency leads to development of metabolic syndrome phenotype, including liver steatosis, hypertriglyceridemia, obesity, and insulin resistance. This study aimed to specifically elucidate the effects of dietary

choline and betaine supplementation in Pcyt2 heterozygous mice (ETKO). Evidence here shows choline and its oxidized metabolite betaine are responsible for lowering whole body weight, restoring insulin sensitivity, reducing hypertriglyceridemia, hepatic steatosis, and alleviating adipose and liver tissue inflammation, by restoring hepatic metabolism and lipogenic gene expression. Collectively, these results establish that the impaired systemic metabolism resulting from Pcyt2 deficiency is a metabolic adaptation that is restored after methyl group supplementation. We further elucidate the mechanism of fatty acids regulation of choline and glycerolipid homeostasis in Pcyt2 knockout mouse liver and skeletal muscle cells. We demonstrate that choline-specific transporter Slc44A1/CTL1 regulates choline requirements for phospholipid synthesis in accordance with the type and availability of fatty acids by regulation of choline Kennedy pathway for phospholipid synthesis.

Very high rates of smoking, low-HDL cholesterol and renal disease among Indigenous Australian adults with poorly controlled diabetes: Implications for primary care and cardiovascular risk 01.07.1415.004

Presenter Last Name: **McDermott**

Background: Indigenous Australians suffer high rates of type 2 diabetes (T2DM), with around 35% of adults affected, and extremely high incidence of preventable vascular complications despite near normal total cholesterol levels. Objective: We aimed to compare the clinical and metabolic profile of a cohort of Indigenous adults with poorly controlled diabetes, to a matched cohort of non-Indigenous adults with T2DM identified from a national sample. Methods: Clinical and sociodemographic data from a cohort of 193 Aboriginal and Torres Strait Islander adults with poorly controlled T2DM (HbA1c>8.5%) was abstracted from medical records and interviews in 2012. National (non-Indigenous) comparator data was obtained from the AusDIAB survey (n=90, matched by HbA1c category). Results: The Indigenous cohort was more than 10 years younger than the national sample (47 vs 58 years), more likely to be female (64% vs 43%), unemployed (47% vs 36%), current smoker (41% vs 16%), more likely to have albuminuria (75% vs 37%) and abnormal renal function (estimated GFR<90 mls/min/1.73m2: 73% vs 46%), suggesting longstanding endothelial dysfunction. There were no differences in BMI. Total cholesterol was significantly lower in the Indigenous cohort than the national sample (4.6, (95%CI=4.4-4.8) mmol/l vs 5.6 (5.3-5.8) mmol/l). Triglyceride, HDL and LDL fraction profile, however, is highly adverse, and accompanied by low rates of statin use. Fatty liver disease is highly prevalent and correlates with high intake of refined carbohydrates and sugars. Conclusions: Indigenous Australian adults with T2DM have an extremely adverse lipid profile despite near normal total cholesterol levels, which is the only lipid fraction required for national reporting for “close the gap” programs. More

attention to appropriate clinical lipid management, nutrition, smoking and other preventable exposures, in addition to early detection and treatment of renal disease, is required.

Relationships between fatty acid status and cardiometabolic health in obese individuals with type 2 diabetes. 01.07.1415.005

Presenter Last Name: **Murphy**

Background –Understanding relationships between dietary factors and cardiometabolic health in individuals with Type 2 Diabetes (T2D) will guide disease management. n-3LCPUFA have been shown to have insulin-sensitising, anti-inflammatory and lipid modulating effects, however the health effects of n-3LCPUFA in T2D remains uncertain. Objective - To explore relationships between fatty acid status and cardiometabolic risk factors in obese men and women with T2D. Design- Cross-sectional analysis was undertaken with erythrocyte fatty acids (% total) and assessments of body composition, blood pressure, fasting blood lipids, insulin, glucose, C-reactive protein (CRP) and glycated haemoglobin (HbA1c) in 61 obese (BMI 34±5kg/m2) men (n=33) and women (n=28) aged 55±8, with T2D. Linear regression analyses were controlled for total energy intake. Outcomes – The O3I and sum of EPA+DPA+DHA of the population was 5.9±2.2% and 8.5±2.7% (mean±SD), respectively. In men, the O3I and total n-3 was negatively related to weight (ß=-0.443, P=0.009; ß=-0.421, P=0.014), insulin (ß=-0.368, P=0.04; ß=-0.388, P=0.029) and CRP (ß=-0.392, P=0.029; ß=-0.355, P=0.05). Total MUFA was positively associated with triglycerides (ß=0.418, P=0.016) and insulin (ß=0.437, P=0.012). Total SFA and the n-6:n-3 was positively related to insulin (ß=0.437, P=0.013 and ß=0.413, P=0.019, respectively). In women, the O3I was negatively related to CRP (ß=-0.512, P=0.009), HbA1c (ß=-0.530, P=0.006) and glucose (ß=-0.527, P=0.006). Total n-3 was inversely related to CRP (ß=-0.518, P=0.009) and HbA1c (ß=-0.532, P=0.006). Total MUFA was positively associated with HbA1c (ß=0.499, P=0.011), glucose (ß=0.547, P=0.005) and CRP (ß=0.493, P=0.013). Total SFA was positively related to HbA1c (ß=0.417, P=0.029) and glucose (ß=0.475, P=0.01) and the n-6:n-3 ratio was positively related to HbA1c (ß=0.504, P=0.008), glucose (ß=0.562, P=0.002) and CRP (ß=0.477, P=0.014). Conclusion - These data suggest that a lower O3I, total n-3, total PUFA status and a higher n-6:n-3 and SFA status, is associated with a poorer cardiometabolic health profile in this population.

Brown fat metabolism and function 01.08.1615.001

Presenter Last Name: **Nedergaard**

The interest in brown adipose tissue metabolism and function has increased markedly since it was established that adult human possess active depots of this tissue. Its functional significance in humans is still not established, but the possibility that it may at least partially be protective against obesity and be a triglyceride- and glucose-clearing tissue makes a further understanding of its functional mechanism important. From rodent experiments we know that the thermogenic and thus energy-dissipating activity of brown adipose tissue is based on the activity of the brown-adipose-tissue-specific uncoupling protein UCP1. The acute regulation of the activity of UCP1 is still not fully understood. Cellular experiments indicate that an increase in the intracellular level of free fatty acids is sufficient to activate UCP1 and that the fatty acids do not have to be metabolized in order to activate UCP1. In isolated brown-fat mitochondria, the activation is seen as a counteraction of the inhibitory effect of cytosolic ATP/GDP on UCP1 activity. Fatty acids thus stimulate mitochondrial respiration on both carbohydrate (pyruvate) and lipid (palmitoyl carnitine) substrate in brown-fat mitochondria. However, fatty acids may act as uncouplers in any type of mitochondria. Thus only by comparing the uncoupling activity in brown-fat mitochondria with and without UCP1 can the characteristics necessary for UCP1 activation be established. Fatty acids may not only affect mitochondrial activity but may also affect mitochondrial structure by altering fusion and fission processes in the mitochondria and they may also influence mitochondrial density and cellular degree of differentiation by interacting with regulatory factors such as PPARgamma. Thus, the regulatory effect of fatty acids may both acutely and chronically affect cellular and thus organismal metabolism.

Brown adipose but not white adipose accumulate DHA in cultured cells provided with alpha-linolenic acid 01.08.1615.002

Presenter Last Name: **Qin**

Introduction. Adipose tissue has recently been recognized as a complex endocrine organ which coordinates numerous crucial biological functions including fatty acid metabolism, glucose metabolism, energy homeostasis, and immune function. White adipose tissue (WAT) stores energy, while brown adipose tissue (BAT) dissipates energy while producing heat. Our aim was to compare the fatty acid profiles of undifferentiated and differentiated WAT and BAT/Beige transformed adipocytes provided with alpha-linolenic acid. Methods. Murine 3T3 L1 fibroblasts (preWAT) or pre-BAT cells were grown in regular media to confluence, and differentiation were then induced to either WAT or BAT. At days 10, 12 and 14, cell were supplemented with 50 μM of albumin bound alpha-linolenic acid (ALA). Cells with no ALA supplementation were additional controls. Twenty-four hours after supplementation, fatty acid profiles of the cells were analyzed by GC and structural mass spectrometry. Results and

DIscussion. ALA supplementation caused an increase in cellular eicosapentaenoic acid (EPA, 20:5n-3) with no change in docosahexaenoic acid (DHA, 22:6n-3) levels in undifferentiated cells. WAT cells accumulated more EPA. Similarly, BAT cells accumulated EPA, however in contrast differentiated BAT cells also accumulate significant amounts of DHA when compared to undifferentiated preBAT cells. BAT adipocytes are characterized by a vast increase in mitochondria which require highly unsaturated fatty acids. DHA may be required for their function. Conclusion. When provided with ALA, WAT cells accumulate EPA with no change in DHA; BATcells accumulate EPA and DHA. If BAT function similarly in vivo, they may serve as a source of DHA for other tissues.

Enhancement of brown fat thermogenesis using chenodeoxycholic acid in mice 01.08.1615.003

Presenter Last Name: **Zouhar**

Objective: Besides their role in lipid absorption, bile acids (BAs) can act as signalling molecules. Cholic acid was shown to counteract obesity and associated metabolic disorders in high-fat diet-fed mice while enhancing energy expenditure through induction of mitochondrial uncoupling protein 1 (UCP1) and activation of non-shivering thermogenesis in brown adipose tissue (BAT). In this study, the effects of another natural BA, chenodeoxycholic acid (CDCA), on dietary obesity, UCP1 in both interscapular BAT and in white adipose tissue (brite cells in WAT) was characterised in dietary-obese mice. Research design: To induce obesity and associated metabolic disorders, male 2-month-old C57BL/6J mice were fed high-fat diet (cHF; 35 % lipid wt wt-1, mainly corn oil) for 4 months. Mice were then fed for either (i) 8 weeks by cHF, or cHF with two different doses (0.5%, 1%; wt wt-1) of CDCA (8-week-reversion); or (ii) 3 weeks by cHF, or cHF with 1% CDCA, or pair-fed (PF) to match calorie intake of the CDCA mice fed ad libitum; mice on standard chow diet were also used (3-week-reversion). Results: In the 8-week-reversion, the CDCA-intervention resulted in a dose-dependent reduction of obesity, dyslipidaemia and glucose intolerance, which could be largely explained by a transient decrease in food intake. The 3-week-reversion revealed mild CDCA-dependent and food intake-independent induction of UCP1-mediated thermogenesis in interscapular BAT, negligible increase of UCP1 in subcutaneous WAT, and a shift from carbohydrate to lipid oxidation. Conclusions: CDCA could reverse obesity in high-fat diet-fed mice, mainly in response to the reduction in food intake, an effect probably occuring but neglected in previous studies using cholic acid. Nevertheless, CDCA-dependent and food intake-independent induction of UCP1 in BAT (but not in WAT) could contribute to the reduction in adiposity and to the stabilization of the lean phenotype. Supported by Czech Science Foundation (13-00871S)

A validation of proposed brown, brite and white adipose marker genes 01.08.1615.004

Presenter Last Name: **de Jong**

Brown adipose tissue (BAT) gained a lot of attention with the acceptance of its presence in adult humans. Its ability to dissipate energy in the form of heat makes BAT a possible target for therapeutics aimed at treating obesity and its comorbidities (e.g. type 2 diabetes). In addition to the 'classical' brown adipocytes, brite (or beige) adipocytes have gained much interest over the past few years. Brite adipocytes are functionally similar to brown adipocytes, but are found interspersed between white adipocytes in what are normally called white adipose tissues (WAT). From a number of studies, a set of genes has been proposed to be suitable to distinguish between 'classical' brown, brite/beige and white adipose tissue. Based on expression of these marker genes, it has been suggested that BAT in adult humans might actually be of the brite type. However, follow-up studies on adult human subjects cannot find a consensus on the identity of human BAT. Here we investigate the validity of the proposed marker genes in a more complete experimental set-up than has previously been done. We performed a parallel expression analysis in interscapular BAT (iBAT), inguinal WAT (ingWAT) and epididymal WAT (eWAT). These tissues are considered the main brown, brite and white adipose depots in mice. In addition, we analyzed expression of the marker genes in primary cell cultures from those depots in the absence and presence of the browning-inducing PPARγ-agonist rosiglitazone. Our results indicate that only few markers seem to properly distinguish between iBAT, ingWAT and eWAT. We suggest to reconsider the use of several of the proposed marker genes and to find alternative ways to distinguish between the different adipose cell types.

Induction of oxidative phosphorylation in white adipocytes: A key to lean phenotype 01.08.1615.005

Presenter Last Name: **Kopecky**

White adipose tissue (WAT) is essential for energy storage, and through its endocrine functions it is also involved in regulation of both glucose and energy homeostasis. Concerning total energy balance, the role of WAT metabolism itself is usually neglected, reflecting a relatively low specific metabolic rate of WAT. However, several pieces of evidence support the notion that energy expenditure in WAT, not mediated by mitochondrial uncoupling, could influence total energy

balance. We focused on a possibility to counteract obesity and associated metabolic disorders by modulating metabolism of WAT. Our results in dietary obese mice demonstrated additive anti-obesity effect of a combined intervention using n-3 fatty acids and calorie restriction, which was associated with increase in (i) mitochondrial biogenesis; (ii) activity of oxidative phosphorylation (OXPHOS); and (iii) activity of a futile substrate cycle based on lipolysis of intracellular triacylglycerols and fatty acids re-esterification (TAG/FA cycle) in epididymal WAT (eWAT). These changes in WAT metabolism were linked with anti-inflammatory effect of the combined intervention (Flachs et al, Diabetologia 2011; Flachs et al, BBA 2013). To further characterize metabolic pathways in WAT, which could be targeted to reduce adiposity, we studied strain-dependent responses to fasting and cold exposure using obesity-resistant A/J and obesity-prone C57BL/6J mice. The new results suggest stronger induction of TAG/FA activity in association with a more pronounced decrease of eWAT weight in the A/J mice, while these strain-dependent effects were not induced in response to fasting. Conclusions: High capacity of mitochondrial OXPHOS linked to inducible TAG/FA cycling activity is essential for metabolic flexibility of WAT, may support leanness, and represents a marker of healthy adipocyte. Treatment of obesity and associated metabolic disorders could be improved by modulating metabolism of white adipose tissue. Supported by Czech Science Foundation (13-00871S) and EU FP7 project DIABAT

Lipid mediators of cutaneous inflammation 01.09.1615.001

Presenter Last Name: **Nicolaou**

The skin produces bioactive skin lipids that participate in its physiological and pathological states including the induction, propagation and resolution of inflammation, and maintenance of normal tissue homeostasis. However, our comprehension of the lipid complement of skin remains incomplete, as well as their contribution to the differing roles of the epidermal and dermal compartments. Lipid mediators derive from membrane fatty acid pools that can be affected by health and nutritional status. In a series of investigations, we have explored the profiles of cutaneous eicosanoids, octadecanoids, docosanoids, endocannabinoids and congeners, and sphingolipids, at baseline and following treatment with inflammatory and irritant stimuli, or systemic interventions with bioactives. We have used tissue and cutaneous blister fluid aiming to explore the prevalence and role of bioactive lipids in the development of inflammation and irritant dermatitis, and understand how precursor skin lipids can affect these. The array of dermal and epidermal fatty acids were reflected in the lipid mediator species produced. Our findings demonstrate the diversity of bioactive lipid mediators that may maintain tissue homeostasis in resting skin, and hint at their contribution to signalling activities, cross-support and functions of different skin compartments. Nutritional interventions may be beneficial in creating an anti-

inflammatory and protective environment that could strengthen cutaneous defences.

Molecular structure determination in situ in native tissues using CEMOVIS combined with electron microscopy simulation

01.09.1615.002

Presenter Last Name: **Norlen**

The skin barrier is fundamental to terrestrial life and its evolution; it upholds homeostasis and protects against the environment. Skin barrier capacity is controlled by lipids that fill the extracellular space of the skin1s surface layer - the stratum corneum. Here we report on the determination of the molecular organization of the skin1s lipid matrix in-situ, in its near-native state, using a novel methodological approach combining very-high magnification cryo-electron microscopy of vitreous skin section (CEMOVIS) defocus-series, molecular modelling and electron microscopy simulation. The lipids are organized in an arrangement not previously described in a biological system – stacked bilayers of fully-extended ceramides with cholesterol molecules associated with the ceramide sphingoid moiety. This arrangement rationalizes the skin1s low permeability towards both water and towards hydrophilic and lipophilic substances, as well as the skin barrier1s robustness towards hydration and dehydration, environmental temperature and pressure changes, stretching, compression, bending and shearing.

Levels of circulating sphingolipids increase with disease severity in psoriasis patients

01.09.1615.003

Presenter Last Name: **Xu**

Psoriasis is a chronic inflammatory skin disorder characterized by the infiltration of inflammatory cells into the epidermis and an impaired keratinocyte differentiation. Ceramides are one of the major compounds in the stratum corneum (SC), the outermost layer of the skin. Human SC contains 12 different free extractable ceramide fractions and most of them have been found altered (either up- or down-regulated) in psoriatic scales. However to date, no studies have shown if these alterations could be reflected in circulating sphingolipids. In this study we quantified 34 NS-class sphingolipids including sphingomyelins, ceramides, glucosylceramides, lactosylceramides and sphingoid bases in plasma from 32 healthy patients (16 male / 16 female) and compared them to patients with mild (n=32) and severe (n=32) psoriasis. Disease groups and healthy individuals were gender balanced. Samples from 16 patients with severe

psoriasis were collected before and after treatment with the TNF-α inhibitor Enbrel to examine treatment effects upon the sphingolipid profile. Circulating levels increased for all the sphingoid bases as well as for most ceramides with disease severity. The other sphingolipid families remained unaltered. Ceramide levels decreased slightly while no changes were observed for sphingoid bases after treatment with Enbrel. The levels of C12:0 sphingolipids decreased in psoriasis patients regardless of the fatty acid chain and recovered after treatment. Additionally, sphingolipids were profiled in skin biopsies from healthy donors and compared it non-lesional and lesional skin from patients. Levels of enzymes involved in sphingolipid biosynthesis were measured by qPCR in keratinocytes, adipocytes, and hepatocytes.. Taken together these data provide the most complete investigation to date of sphingolipid metabolism in psoriasis.

Inflammation induced by solar radiation and facial preadipocytes 01.09.1615.004

Presenter Last Name: **Pappas**

The loss of facial subcutaneous fat is associated with aging. Facial preadipocytes (FpAd) were isolated, differentiated and characterized in relation to abdominal preadipocytes. Fatty acid related genes transcriptional analysis was performed and in relationship to inflammatory cytokines which are known to inhibit the differentiation process of preadipocytes. Differences in adrenergic receptors were identified, which further implicates fatty acid catabolism. The potential role of inflammatory mediators in solar-radiation-induced facial fat loss was investigated. Cultured fibroblasts, keratinocytes, and 3D-skin-equivalents were exposed to various doses of radiation by using a solar simulator. Inflammatory cytokines were examined by qRT-PCR and ELISA. Epidermal-dermal equivalents were also treated topically with a broad-spectrum sunscreen prior to solar simulated radiation (SSR). Human FpAd treated either with recombinant IL-11 or with conditioned media from solar-irradiated equivalents and were later evaluated for the level of adipocyte differentiation by Oil red O staining and the expression of adipocyte markers. Interestingly FpAd responded differently to adipogenic and lipolytic factors than the abdominal cells. IL-11, IL-1α, IL-6, and TNF-α protein secretion were induced from epidermal-dermal equivalents by exposure to SSR; however sunscreen pretreatment prevented the SSR-induced inflammatory cytokines production from such equivalents. Exposure of FpAd to conditioned medium from solar-irradiated epidermal-dermal equivalents inhibited their differentiation however conditioned medium from sunscreen-pretreated, solar-irradiated equivalents did not inhibit differentiation of facial preadipocytes. In addition, a cocktail of neutralizing antibodies to IL-11, IL-1α, IL-6 and TNF-α significantly reduced the SSR-induced inhibition of facial preadipocyte differentiation. These results support the hypothesis that SSR-induced inflammatory cytokine production may be involved

in the photoaging-induced loss of facial subcutaneous fat and that inhibition of this process might slow or prevent photoaging-induced changes.

California Sea Lions Have Vernix that Deliver Branched Chain Fatty Acids to the Fetal Gut 01.09.1615.005

Presenter Last Name: **Ran-Ressler**

Introduction Vernix caseosa has long been thought to be a uniquely human substance, not demonstrated in any other species. Monomethyl branched chain fatty acids (BCFA) synthesized by the sebaceous glands are at uniquely high concentration in vernix and serves as a marker for skin lipids. Amniotic fluid-borne vernix particles appearing in the last third of human gestation are swallowed by the fetus and deliver BCFA throughout the GI tract where they appear in meconium at birth. We found physical vernix on the skin of newborn California sea lions born in captivity, and collected amniotic fluid, gastric contents, serum, and meconium. Our aim was to determine whether California sea lions have physical vernix resembling humansthat can serve as a vehicle for BCFA delivery to the fetal gut. Methods. Skin surface debris present at delivery(vernix), amniotic fluid, serum, gastric contents, and meconium were collected from five California sea lions at birth and analyzed for BCFA structure and profile. Results. California sea lions have a white waxy vernix-like material on their skin. Amniotic fluid, vernix, gastric contents, serum, and meconium had 18%, 5%, 3.6%, 1.2%, and 12% by w/w BCFA. BCFA chain lengths were from C15 to C24. iso-BCFA dominated with iso-20:0, iso-22:0, and iso-18:0 as the highest in most samples. anteiso-BCFA totaled about 0.5%. Total meconium BCFA of 12% is more similar to the human level of about 17% than to other dog and piglet meconium BCFA which were <1%. The serum BCFA suggests that some is absorbed. Conclusion. California sea lions are born with a vernix like material with much lower BCFA concentration than humans (29%), however meconium BCFA are similar to humans suggesting that vernix delivers BCFA to the fetal gut in a manner similar to humans.

Manipulation of lipids in animal-derived foods: can it contribute to public health nutrition? 01.10.1615.001

Presenter Last Name: **Givens**

In spite of the recognised benefits of replacing saturated fatty acids (SFA) in the diet with cis-PUFA/MUFA few parts of the EU meet recognised targets. Milk and

dairy products represent the single largest source of dietary SFA in most countries yet epidemiological evidence indicates that milk has cardioprotective properties such that simply reducing consumption of dairy foods to meet SFA targets may not be a sound public health approach. This paper explores the options for replacing some of the SFA in milk fat with cis-MUFA through alteration of the diet of the dairy cow and the evidence that such changes can improve markers of CHD/CVD risk in the consumer. For sustainability reasons there is increasing interest in feeding ruminants on diets containing more fresh grass. This has modifying effects on milk and meat lipids with higher concentrations of n-3 fatty acids and phytanic acid (PA). In vitro studies have shown PA can improve glucose handling and stimulate fatty acid oxidation athough further work is needed. The beneficial effects of long chain (LC) (carbon chain ≥20) n-3 PUFA are well documented but recent evidence indicates that few people achieve the UK daily recommended intake for adults of 450 mg of EPA + DHA per day. In many parts of Europe the daily intake of EPA + DHA by adults and especially young people is less than 100 mg per day, since many never eat oily fish. There is also concern that intake of n-6 PUFA has increased excessively and is aggravating the effects of low LC n-3 intake. Poultry meat contributes small but worthwhile amounts of EPA + DHA and studies to enrich the EPA + DHA content of animal-derived foods will be described and how this would impact on habitual intake. Research is however required to characterise the benefits associated with lipid-modified foods and to understand how the active compounds in natural foods can be enhanced. In the future, the role of animal nutrition in creating foods closer to the optimum composition for chronic health is likely to be more important but production of such foods on a scale that will substantially affect national diets will require political and financial incentives and great changes in the agro-food industry.

Toxic salmon membranes? Potential effects on biomembranes of polyaromatic hydrocarbons studied using model systems

01.10.1615.002

Presenter Last Name: **Liland**

During the last decade, the diet of aquacultured Atlantic salmon (Salmo salar L.) has changed from being based on marine ingredients to containing increasing amounts of plant ingredients, due to limited availability of marine feed ingredients. This has altered the amount and composition of contaminants in the feed, i.e. the amount of marine contaminants decrease whereas contaminants from vegetable oils are being introduced (Berntssen et al. 2010). Polyaromatic hydrocarbons (PAH) are toxic substances found in many seed oils due to the thermal processing of the seeds, and may thus be present in modern salmon diets. One of the possible cytotoxic modes of actions of PAH is a perturbation of the membrane structure (Schirmer et al. 1998). A solid-supported lipid bilayer

(DOPC:DPPC:cholesterol) was used to investigate the effects of three different PAH (benzo-α-pyrene, phenanthrene and naphthalene) on the formation of liquid-ordered (lo) and liquid-disordered (ld) domains using fluorescence microscopy. It was demonstrated using the native fluorescence of the PAH compounds, that they incorporate preferentially into the lo phase of a phase separated membrane. A calorimetric analysis of each PAH in DPPC was also performed. No clear effects of PAH on the size of the domains or on the ratio between lo and ld were seen. Adding PAH to DPPC did also not alter the gel phase transition temperature. Apart from incorporation, we could thus not detect any PAH-induced changes in lipid bilayer properties. Berntssen MHG, Julshamn K & Lundebye A-K (2010) Chemical contaminants in aquafeeds and Atlantic salmon (Salmo salar) following the use of traditional- versus alternative feed ingredients. Chemosphere 78, 637-646. Schirmer K, Dixon DG, Greenberg BM et al. (1998) Ability of 16 priority PAHs to be directly cytotoxic to a cell line from the rainbow trout gill. Toxicology 127, 129-141.

Use of high-EPA oil from transgenic Camelina sativa in feeds for aquaculture 01.10.1615.003

Presenter Last Name: **Betancor**

The beneficial effects that dietary omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) have on human health are widely known. Fish are the major source of n-3 LC-PUFA in the diet and, with declining fisheries worldwide, farmed fish and seafood now represent around 50 % of consumption. However, feeds for farmed fish have been traditionally based on fishmeal and fish oil themselves derived from marine fisheries that are also at sustainable limits. Therefore, vegetable oils have increasingly replaced fish oils in aquafeeds, resulting in reduction of n-3 LC-PUFA levels and, potentially, nutritional quality of farmed products. One approach to the renewable supply of n-3 LC-PUFA is the metabolic engineering of oilseed crops with the capacity to synthesize these essential fatty acids. Recently, the oilseed Camelina sativa was transformed with algal genes encoding the n-3 biosynthetic pathway and expression restricted via seed-specific promoters to produce an oil containing >20% eicosapentaenoic acid (EPA). The aims of the present study were to investigate the replacement of fish oil by this high-EPA oil in aquafeeds and to gain insight into the biochemical and molecular mechanisms involved in the control and regulation of docosahexaenoic acid production from EPA in fish. Three diets with either fish oil (FO), wild-type Camelina oil (WCO) or EPA-Camelina oil (ECO) as sole lipid sources were formulated and fed to Atlantic salmon (Salmo salar) post-smolts for 7 weeks. Fish from all treatments more than doubled their weight and no mortality was recorded. Salmon fed the ECO diet showed the highest growth in terms of weight gain compared to fish fed the FO diet. Tissue transcriptomic

responses and biochemistry were determined in order to assess the influence of the experimental feeds on fish metabolism and health.

Potential for production of Atlantic salmon families with improved capacity for EPA and DHA production 01.10.1615.004

Presenter Last Name: **Berge**

The main lipid source in feeds for Atlantic salmon has traditionally been fish oils. The amount of fish oils available on the world market is however not sufficient to meet the requirements from a growing aquaculture industry. Increasing levels of plant oils are therefore used in fish diets, with a reduction of EPA and DHA in the salmon fillets as a consequence. The aim of this project was to increase the innate ability of salmon to produce EPA and DHA from α-linolenic acid by genetic selection and optimization of dietary fatty acid composition, in order to secure the nutritional quality of salmon for human consumption. Atlantic salmon individuals were randomly sampled from 103 SalmoBreed families. Gene expression of delta-6-desaturase (D6fad_b) in liver was measured in approximately 800 fish, Gene expression of D6fad_b in liver, varied from 0.15 to 3.37 between families and parent fish with high or low expression of D6fad_b were selected to produce first generation offspring. 3 “high” and 3 “low” offspring families were fed different dietary levels of fish oil vs rapeseed oil. EPA and DHA levels in whole body lipids was measured in fish at start (95g) and end of the feeding trial, when the fish was approximately 400g. Offspring from the high D6fad_b families had slightly higher muscle levels of EPA and DHA. These fish had also higher DHA deposition rate in whole body lipids than the low D6fad_b families, in particular in fish fed moderate dietary levels of rapeseed oil. Further, the dietary plant oil level influenced the gene expression of desaturases and elongases differently in ”high” and “low” families. The variation between Atlantic salmon families in capacity to produce EPA and DHA shows a potential for genetic selection of this trait.

Heterologous synthesis of omega-3 long chain polyunsaturated fatty acids in transgenic plants via iterative metabolic engineering: a terrestrial source of fish oils 01.10.1615.005

Presenter Last Name: **Napier**

We have been evaluating the possibility of producing omega-3 long chain polyunsaturated fatty acids (LC-PUFAs) in different transgenic hosts, to provide a sustainable source of these important nutrients. Attempts to metabolically engineer plants with the primary biosynthetic pathway for LC-PUFAs has been carried out in both model plants and crop species, allowing insights into factors

constraining the accumulation of these otherwise non-native fatty acids. Specifically, a generic bottleneck resides within the primary LC-PUFA biosynthetic pathway as a result of the “substrate dichotomy” between the lipid-dependent desaturases and the acyl-CoA-dependent elongases which catalyze the primary reactions. This bottleneck can be overcome through the use of acyl-CoA dependent desaturase, though not without impact on phospholipid composition. The use of lipidomic analyses have allowed us to identify further interventions in this pathway, ultimately leading to the breakthrough production of a transgenic oilseed crop which contains up to 30% omega-3 LC-PUFAs in seed oil. The practical challenges associated with moving these complex engineered traits out of the lab and into the field will also be discussed. Our ultimate goal is to ensure an alternative, sustainable source of EPA and DHA.

Liver X receptor β – a multifunctional ligand activated transcription factor 01.11.1615.001

Presenter Last Name: **Gustafsson**

Liver X receptor (LXR) β is a later discovered member of the nuclear receptor (NR) gene family. LXRβ is an oxysterol-dependent NR that controls lipid homeostasis, water transport, immune response and neural development. The motor neurons of the spinal cord, the dopaminergic neurons of the substantia nigra (SN) and the neurons of the prefrontal cortex depend on the presence of LXRβ. This receptor is involved in amyotrophic lateral sclerosis and Parkinson’s disease (PD). At present there is no specific LXRβ agonist available so we have worked with LXRβ-/- mice and an agonist which acts on both LXRα and LXRβ. Male but not female LXRβ-/- mice develop motor neuron disease as they age. The first symptoms occur when the mice are 6 months of age and begin to perform poorly on the rotor rod. The disease progresses to paralysis when mice are one year old and is accompanied by loss of motor neurons and dopaminergic neurons. Administration of the LXR agonist, GW3965, protects mice against the MPTP-induced loss of dopaminergic neurons in wild type (WT) mice. Thus LXR agonists may have beneficial effects in treatment of PD. LXRβ may be target for treatment of various forms of neurodegeneration by modulating the cytotoxic functions of microglia. NRs are multidomain transcription factors. Different NRs bind to a multitude of DNA response elements. The nature of the conformational adaptations NRs undergo to bind to DNA is incompletely understood. The structure will be presented of a heterodimer of human LXRβ and retinoid X receptor (RXR) α upon a direct repeat (DR-4) AGGTCA DNA element with low molecular ligands and cofactor peptides. The complex has LXRβ in the 3’ half-site and DBDs and LBDs crossed to place the LXRβ LBD in the 5’ position. Evidence is presented for conformational flexibility within the NR family.

Urinary LTE4 is a new strong predictor of TH2-driven asthma: Initial data from the Pan-European U-BIOPRED IMI project

01.11.1615.002

Presenter Last Name: **Kolmert**

Background: Lipid mediators (e.g. eicosanoids) contribute to the pathobiology of asthma and other respiratory diseases. We hypothesised that urinary lipid mediator profiles could be used as biomarkers to identify specific phenotypes of asthma. We therefore determined the urinary concentrations of the main metabolites of the cysteinyl-leukotrienes (CysLTs), prostaglandins (PGs) and isoprostanes (IPs) in the U-BIOPRED study (Unbiased BIOmarkers in PREDiction of respiratory disease outcomes) of severe asthma (SA). Methods: Subjects (aged 18-79) with severe asthma (SA; n=231), controlled mild asthma (MA; n=71) and healthy controls (HC; n=72) were recruited according to internationally recognized criteria (Chung et al Eur Resp J 2014). Spot urine samples were analysed using a published UPLC-MS/MS platform (Balgoma et al Anal Chem 2013) including 13 different main lipid mediators indicative of whole body formation of CysLTs, PGs and IPs. Optimal extraction volumes for solid phase extraction were calculated using individual UV absorbance (λ=300 nm) measurements to minimize matrix effects. Results: The urinary excretion of all lipid mediators, except tetranorPGEM, were highest among the subjects with SA, and generally higher in MA vs. healthy controls, - with LTE4 the most significant (MFC (median fold change)=2.1 for SA vs. HC, P<0.001). The markers of oxidative stress, isoprostane 8-iso-PGF2α (MFC=1.6, P=0.001) and 2,3-dinor-8-iso-PGF2α (MFC=1.3, P=0.01), displayed a particularly pronounced increase in the subgroup of smokers with SA (n=35). Prediction accuracy of SA and HC validation samples in a multivariate OPLS-DA model was high (87% overall, 91 % for SA and 72 % for HC) with descending contribution of lipid mediators; LTE4>tetranorPGDM>2,3-dinor-11-B-PGF2α >8-iso-PGF2α>2,3-dinor-8-iso-PGF2α >11-dihydroTXB2. Conclusion: Increased urinary LTE4 levels correlated with clinically significant depression of baseline lung function and a Th2 profile of airway inflammation. The univariate and multivariate data supports that monitoring of key urinary lipid mediators may be helpful to identify subgroups of asthmatics for stratification of treatment.

EFFECT OF SHORT-TERM N-3 FATTY ACID SUPPLEMENTATION AND ASPIRIN ON CIRCULATING RESOLVINS IN HEALTHY VOLUNTEERS 01.11.1615.003

Presenter Last Name: **Mori**

Background: Inflammation is associated with many diseases. Resolution of inflammation is an active process involving specialised proresolving mediators

(SPM) formed from the n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). SPM are derived from lipoxygenase, cyclooxygenase (COX-2) or cytochrome P450 actions on EPA and DHA. Aim: This study aimed to examine the effect of n-3 fatty acid supplementation and aspirin on plasma SPM derived from EPA and DHA in a placebo controlled trial in healthy humans. Methods: Healthy men and women (n=21) were supplemented with 2.4g/day of n-3 fatty acids for 7 days. After 5 days the volunteers were randomly assigned to take either aspirin (300mg/day) or placebo for 2 days. Blood samples were obtained at baseline day 5 and day 7 for measurement of the EPA derived E-series resolvins and their precursor 18-HEPE, the DHA derived D-series resolvins and their precursor 17-HDHA, and SPM of the maresin family including 14-HDHA and MaR1, using liquid chromatography tandem mass spectrometry. Results: At baseline concentrations of E- and D-series resolvins and the upstream precursors 18-HEPE, 17-HDHA ranged from 0.1-0.2nM. 14R/S-HDHA was threefold higher than the other SPM at baseline but we were unable to detect maresin1. Supplementation with n-3 fatty acids significantly increased RvE1, 18-HEPE, 17-HDHA and 14-HDHA, but not other SPM. The addition of aspirin after 5 days of n-3 fatty acids did not affect concentrations of any SPM although it altered the ratio of the R:S isomers of 17-HDHA. Conclusion: The observed increase in RvE1 and 17-HDHA with n-3 fatty acids in healthy humans is important because both of these compounds have been shown to be biologically active. Aspirin did not appear to offer any additional benefit in elevating the levels of SPM after fish oil in healthy humans.

The Novel Lipid Mediator PD1n-3 DPA: Structural Elucidation, Biosynthesis, Bioactions and Total Organic Synthesis

01.11.1615.004

Presenter Last Name: **Hansen**

The Novel Lipid Mediator PD1n-3 DPA: Structural Elucidation, Biosynthesis, Bioactions and Total Organic Synthesis Trond V. Hansen,a,b Marius Aursnes,a Jørn E. Tungen,a Anders Vik a Romain Colas,b Chien-Yee C. Cheng,b Jesmond Dallib and Charles N. Serhanb aSchool of Pharmacy, Department of Pharmaceutical Chemistry, University of Oslo, PO Box 1068 Blindern, N-0316 Oslo, Norway. bCenter for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Harvard Institutes of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, 02115. Several novel lipid mediators families coined specialized pro-resolving mediators (SPMs) are formed during the resolution phase of acute inflammation in animal models of self-limited inflammation. The SPMs are biosynthesized from the dietary n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The resolvins, protectins and maresins are examples of such SPMs. In

2013 Dalli, Colas and Serhan reported a new SPM that was coined PD1n-3 DPA.1 This C22 n-3 oxygenated SPM is biosynthesized from n-3 docosapentaenoic acid (n-3 DPA) that can accumulate in humans. In this presentation, the structural elucidation and the biosynthetic pathway, together with the potent anti-inflammatory and pro-resolving properties of the PD1n-3 DPA, will be presented. The first total organic synthesis will briefly also be outlined. The results presented contribute new knowledge on the structure-function of the growing numbers of endogenous novel SPMs. The authors declare no conflict of interests. 1. Dalli, J.; Colas, R. A.; Serhan, C. N. Sci. Rep. 2013, 3, 1940, DOI: 10.1038/srep01940

Non-redundant Transcriptional Target for Cell Survival of the Essential Docosahexaenoic Acid-derived Mediator Neuroprotectin D1 01.11.1615.005

Presenter Last Name: **Bazan**

Neuroprotectin D1 (NPD1) induces cell survival in uncompensated oxidative stress, neurodegenerations or ischemic stroke. The molecular principles underlying this protection remain unclear. We found that, in retinal pigment epithelial cells, NPD1 triggers nuclear translocation and cREL expression that, in turn, mediates BIRC3 synthesis. NPD1 induces NF-κB by an alternate route to canonical signaling, and the opposing effects of TNFR1 and NPD1 on BIRC3 expression likely are not due to interaction/s between NF-κB pathways. Our data suggest that cREL regulates a cluster of NPD1-dependent genes after cREL nuclear translocation. BIRC3 silencing prevents NPD1 induction of survival against oxidative stress, suggesting non-redundancy. Moreover, brain NPD1 biosynthesis and selective neuronal BIRC3 abundance are increased by DHA after experimental ischemic stroke followed by remarkable neurological recovery. Thus, NPD1 bioactivity is a decision-making event that counter-regulates key gene transcription steps for neural cell integrity when confronted with potential disruptions of function.

Fatty acid desaturase 2 (FADS2). Not just a 6-desaturase anymore. 02.12.1000.001

Presenter Last Name: **Brenna**

Introduction. Conversion of C18 polyunsaturated fatty acid (PUFA) into highly unsaturated fatty acids (HUFA) is normally depicted as a competitive, linear biochemical pathway, in which 6-desaturation is often referred to as rate limiting and existing only in the endoplasmic reticulum (ER). Molecular, isotope tracer,

and specific structural mass spectrometry techniques reveal that the gene product of fatty acid desaturase 2 (FADS2, 11q12.2) has at least seven, and probably nine, fatty acid substrates. Old and more recent data support either desaturase activity or desaturase protein in the nuclear membrane, or in mitochondria. Our aim is to review the well known substrates for which FADS2 catalyzes 6 and 8 desaturation, and present original evidence that FADS2 4-desaturates 22:5n-3 and 22:4n-6.

Methods. FADS2 protein has long been believed to 6-desaturate 18:2n-6, 18:3n-3, 16:0(→16:1n-10), 24:4n-6, and 24:5n-3. Yeast and a human breast cancer cell line lacking 6-desaturase activity, MCF7 cells, are our primary in vitro models. Transfection of FADS2, but not FADS1, gains activity toward all these substrates, while FADS1 transfection causes gain of 5-desaturase activity toward 20:3n-6 and 20:4n-3. Yeast transformed with FADS2 gain activity toward 20:2n-6 and 20:3n-3 to yield 20:3n-6 (DGLA) and 20:4n-3, both eicosanoid precursors. Stably transfected MCF7(FADS1) and MCF7(V) cells incubated with 20:2n-6 and 20:3n-3 synthesize 5,11,14-20:3 and 5,11,14,17-20:4, respectively, presumably by action of native and transfected FADS1, similar to FADS2-null mice and wild type FADS2-null cats in vivo. MCF7(FADS2) cells have normal 6 and 8 desaturase activity. Both these results confirm that the cell model accurately recapitulates in vitro and in vivo biology. We studied stably transfected FADS2, FADS1, and vector-only (V) MCF7 cells for their kinetics and dose response with emphasis on C20-24 PUFA.

Results and Discussion. All MCF7 cells incubated with 22:6n-3 or 22:5n-6 accumulate, respectively, 24:6n-3 and 24:5n-6 in a dose response manner indicating that both substrates are elongated and therefore are precursors of the respective C24 PUFA. MCF7(FADS2) cells incubated with 22:4n-6 accumulate 22:5n-6 before any 24:5n-6 is detected; if 24:5n-6 is transported from ER to peroxisomes, it should be detectable before or at least concomitant with 22:5n-6. Similarly, MCF-7(FADS2) cells incubated with 20:5n-3 (EPA) or 22:5n-3 accumulate 22:6n-3 (DHA). When incubated with d5-18:3n-3, MCF7(FADS2) accumulate d5-22:6n-3 several hours before any label can be detected in 24:5n-3 or 24:6n-3. Data of others have established that the last step in DHA synthesis in two vertebrate fish species and in single celled dinoflagellates, consistent with the present data. Strong attenuation of rodent tissue DHA accumulation by high 18:2n-6 feeding ascribed to double use of FADS2 is consistent with competition of 18:2n-6 for FADS2 mediated 4-desaturation, as well as the presumed 6-desaturation of 24:5n-3. We conclude that FADS2 mediates desaturation at three (4, 6, 8) positions, specific to each of eight different PUFA and one saturated fatty acid.

A low omega-6 polyunsaturated fatty acid (n-6 PUFA) diet increases omega-3 (n-3) long chain PUFA status in plasma phospholipids in humans 02.12.1000.002

Presenter Last Name: **Wood**

Background: The competition between omega-6 and omega-3 polyunsaturated fatty acids (n-3 and n-6 PUFA) for both metabolism and incorporation has led to suggestions that reducing dietary n-6 PUFA intake would improve n-3 PUFA status without the need to increase current intake. Aim: To determine the effect of reducing the linoleic acid (LA) content of the diet from ~5% to <2.5% energy (%E) for 4 weeks on n-3 long chain polyunsaturated fatty acid (LCPUFA) status in healthy human subjects. Methods: Thirty-six participants (n= 12 males, 24 females) followed a diet with a target LA intake of <2.5%E (low LA diet) for 4 weeks. Nutrient intakes were estimated from diet diaries maintained for 2 weeks before and throughout the low LA intervention and blood samples were collected before and after the low LA diet phase for assessment of fatty acid composition in plasma and erythrocyte phospholipids. Results: LA intakes were reduced from 4.6%E at baseline to 2.0%E during the intervention phase ($P<0.001$) with no change in the intake of n-3 LCPUFA. The LA content of both plasma and erythrocyte phospholipids were significantly reduced at the end of the low LA diet phase ($P<0.001$), but AA levels in both fractions were unchanged. The n-3 LCPUFA content was increased in plasma phospholipids at the end of the low LA diet phase compared to baseline (6.22% vs 5.53%, $P<0.001$). Conclusion: These data demonstrate that reducing dietary LA intake for a 4 week period can increase the n-3 LCPUFA status in healthy humans in the absence of any increase in dietary n-3 LCPUFA intake above normal Australian levels. Further studies to assess the longer-term effects of low n-6 PUFA diets on clinical outcomes are warranted.

Transgenic mice convert carbohydrates to essential fatty acids: Implications for modern health epidemics 02.12.1000.003

Presenter Last Name: **Kang**

The worldwide trend of increased saturated fat and carbohydrate consumption with increased omega-6 (n-6) and decreased omega-3 (n-3) fatty acid intake has coincided with the growing prevalence of chronic, life-threatening diseases, suggesting a critical link between the shift in our dietary composition and today's health epidemic. Saturated fats can be readily produced from carbohydrates in mammals, and both saturated fats and carbohydrates are highly abundant in the modern Western diet; in contrast, omega-3 fatty acids cannot be made de novo nor converted from other nutrients in mammals, and therefore must be obtained

from dietary sources (mainly fish oil). Here we show that mice engineered to carry both fat-1 and fat-2 genes from the roundworm Caenorhabditis elegans (named “Omega mice”) are capable of producing essential n-3 fatty acids from saturated fats or carbohydrates. When maintained on a high-fat diet lacking essential fatty acids or a high-carbohydrate, no-fat diet, the Omega mice exhibit high tissue levels of both n-6 and n-3 fatty acids, with a ratio of ~1:1. Furthermore, when the three genotypes generated in this study – wild-type (incapable of producing essential fatty acids), fat-2 transgenic (producing only n-6 and no n-3 fatty acids), and Omega transgenic (producing both n-6 and n-3 fatty acids) – were fed the same diet, they presented distinct profiles of hepatic lipogenesis, gut microbiota, and low-grade inflammation, all of which have been implicated in many metabolic diseases. Our discovery thus provides a novel tool for addressing fat metabolism and disease, as well as an innovative technology for the production of both n-6 and n-3 essential fatty acids.

Victor J. Pai*, Bin Wang*, Xiangyong Li, Kanakaraju Kaliannan, Yuan Sun, Yinghua Liu, Marina Kang, Chih-Yu Chen, Hongman Zhang, Amy Goodale, Lorenzo Ramos-Mucci, Lin Wu, Jing X. Kang# Massachusetts General Hospital and Harvard Medical School, Boston, MA 02129

Dietary supplementation with fish or olive oil induces altered DNA methylation at specific CpG loci in FADS2 in adult humans with renal disease 02.12.1000.004

Presenter Last Name: **Burdge**

Long chain polyunsaturated fatty acid (LCPUFA) biosynthesis is modified by the transcriptional activity of genes that encode key enzymes in this pathway, including FADS2 which encodes Δ6-desaturase. A high fish oil (FO) diet induced hypermethylation and lower expression of the Fads2 promoter in rat liver. We investigated the effect of dietary supplementation with FO or olive oil (OO) on the DNA methylation of the FADS2 promoter in leukocytes from men and women with renal disease. Subjects (56±1y) received either FO or OO (4g/d) for 8 weeks. DNA was prepared from leukocytes collected at baseline or study end. Methylation of 19 consecutive CpGs between -18 to -1337bp relative to the transcription start site in the FADS2 promoter was measured by pyrosequencing and mRNA expression by qRTPCR. DNA methylation was not associated significantly with potential confounders; age, body-mass-index, insulin resistance or leukocyte numbers. In the OO group, compared to baseline methylation of CpGs -1119 and -1112 was 5% higher and of 6 CpGs between -871 and -775bp ≥10% higher (all negatively correlated with mRNA expression (β≥ -0.544, $P<0.05$)) in women (n=7). CpGs -806 and -775 were hypermethylated (14% and 13%, respectively), and both associated negatively with expression (β≥ -0.69, $P<0.05$), in men (n= 11). In the FO group, methylation of CpGs -1071, -975, -871 and -775 were ≥5% higher compared to baseline, all negatively associated with

expression (β ≥-0.49, P<0.05), in women (n=7). CpG -806 was hypermethylated (13%) and negatively correlated with expression (β=-0.38, P<0.05), and CpG -775 was hypomethylated (6%), but not associated with expression, in men (n= 8). These findings show that in adult humans dietary oils induce differential changes in the methylation of individual CpG loci in the FADS2 promoter, which are contingent on sex. This represents a possible mechanism by which the dietary fat may modulate LCPUFA biosynthesis.

FADS polymorphisms and fatty acid composition in blood at age 2, 6 and 10 years. 02.12.1000.005

Presenter Last Name: **Standl**

Background Polyunsaturated fatty acids (PUFAs) have a major impact on health and development. The desaturase enzymes, which regulate PUFAs conversion, are encoded by the FADS genes. Minor allele carriers exhibit increased levels of desaturase substrates and decreased levels of desaturase products. We investigated whether the effect of FADS gene variants on PUFA composition in blood, measured at three different time points, is consistent from 2 to 10 years of age. Methods Fatty acids with 14 to 24 carbon atoms were measured in serum collected at 2, 6 and at 10 years of age by gas chromatography in 1097 children from the Munich LISAplus birth cohort study. Three FADS polymorphisms (rs174556, rs3834458 and rs174575) were genotyped. The effects of these FADS polymorphisms on the percentage of seven and four metabolites in the n-6 and n-3 PUFA pathways, respectively, were analysed using linear mixed models including an interaction between FADS variant and time and assuming an additive genetic effect. Results All investigated PUFAs were significantly associated with at least one of the three FADS variants. Time showed a significant effect on all PUFAs, except for C22:5n-3. Additionally, for arachidonic acid (C20:4n-6), there was a significant interaction effect between rs174556 and rs3834458 with time, showing a stronger effect of the FADS variants with increasing age. For all other PUFAs, no significant SNPxtime interaction was observed. Conclusion The three FADS variants investigated were consistently associated with PUFA composition in blood collected at 2, 6 and 10 years of age. Only for arachidonic acid, the effect of the FADS variants was stronger with increasing age.

Fish oil and krill oil supplementation differentially regulate lipid metabolism in the mouse 02.12.1000.006

Presenter Last Name: **Alexson**

Fish oil (FO) have long been associated with several health promoting effects, such as reduced plasma lipid levels and anti-inflammatory events, ascribed to the content of the long-chain omega-3 fatty acids EPA (eicosapentaenoic acid, 20:5) and DHA (docosahexaenoic acid, 22:6). Krill oil (KO) is also rich in EPA and DHA, but with the majority of the omega-3 PUFAs being incorporated into phospholipids rather than triglycerides. This study investigates the effects of supplementation with 6% FO or KO to mice fed a high fat diet. Although the total omega-3 fatty acid content in the two oils were different (19% and 10% of total fatty acids in the FO and KO diets respectively) this difference was not reflected in the plasma content of total omega-3 fatty acids, which were significantly elevated to the same level in the two marine oil supplemented groups. Plasma cholesterol (total, free and esterified), triglycerides and total phospholipids were significantly decreased in the FO supplemented group, which were accompanied by increased levels in liver. These effects were weaker in the KO group, however, KO significantly lowered plasma levels of non-esterified fatty acids. FO and KO increased omega-3 fatty acids in the phospholipid fraction in liver to the same extent, but the omega-3 fatty acid content was markedly higher in the triglyceride fraction in the FO group. The increased liver lipid content in the FO group was unexpected in view of that expression of most genes involved in lipid catabolism were elevated in a peroxisome proliferator activated receptor alpha (PPARα) activation pattern in livers from FO fed mice. This pattern was much less pronounced in the KO group in which the expression of several lipogenic and cholesterol synthesizing genes were decreased at mRNA level. In conclusion, FO and KO regulate lipid homeostasis via partially different mechanisms.

A role for the group-II secreted phospholipase A2 in the establishment of lung microbiome in patients with cystic fibrosis

02.13.1000.001

Presenter Last Name: **Touqui**

Cystic fibrosis (CF) is a lethal autosomal, recessive inherited disease that commonly affects Caucasians. This disease is due to mutation of CF transmembrane conductance regulator (CFTR) gene that encodes a protein channel in epithelial cells where it regulates the luminal secretion of chloride and water transport. The F508del-CFTR is the most frequent mutation in CFTR present in CF patients. Pulmonary disease in CF is the major problem that determines the life span and life quality of patients and contributes to 80-95% death of CF patients. In the lungs, mutations of CFTR cause depletion of airway surface liquid and mucus dehydration, which provide appropriate niche for chronic bacterial infection by opportunistic pathogens. Bacterial airways infection varies significantly with the age of patients and *Staphylococcus aureus* (SA) is

the most commonly isolated bacterium from young CF patients. With the increase of age of patients, *Pseudomonas aeruginosa* (PA) becomes predominant and in the adult patients represents over 80% of bacteria in the lung. However, the mechanisms responsible for this age-related infection switch from SA to PA remained unclear.

We postulated that a selective elimination of SA in CF airways by a host molecule endowed with antibacterial activity might be involved in the infection switch from SA to PA. Among the host molecules endowed with antibacterial properties, antimicrobial peptides (AMPs) play a key role in natural host defense toward invading pathogens. AMPs are ubiquitous, gene-encoded natural "antibiotics" including cationic peptides, defensins and some phospholipase A2 (PLA2s). PLA2s represent a family of mammalian enzymes, which hydrolyzes membrane phospholipids of eukaryotic and prokaryotic cells leading to the release of fatty acids. This hydrolysis participates in a variety of processes such as inflammation. Among mammalian PLA2s, group-IIA secreted PLA2 (sPLA2-IIA) has been shown to exert potent bactericidal effect. Gram-positive bacteria are much more sensitive to sPLA2-IIA than Gram-negative bacteria due to higher affinity of sPLA2-IIA to the cell walls of Gram-positive bacteria. Selective hydrolysis of bacterial membrane phospholipids represents the critical step of bactericidal action of sPLA2-IIA. This enzyme is the most potent bactericidal agent produced by mammals and is, for example the unique bactericide for *S. aureus* in human tears.

Our recent studies showed that sPLA2-IIA levels increased in expectorations of CF patients in age-dependent manner. These levels were sufficient to kill SA with only marginal effects on PA strains. Bronchial epithelial cells (BECs) are major cell source of this enzyme in CF patients. Both laboratory strains and PA isolates from CF patients induced sPLA2-IIA expression by BECs from CF patients. In animal model of lung infection, PA induced sPLA2-IIA production that favors SA killing. We suggest that sPLA2-IIA induction by PA contributes to SA eradication in CF airways. This highlights a new concept suggesting that a bacterium can eradicate another bacterium by manipulating the host immunity. In this talk we present the molecular mechanisms by which PA induces sPLA2-IIA expression by host cells. Erwan PERNET*, Laurent GUILLEMOT*, Pierre-Regis BURGEL, Clemence MARTIN, Isabelle SERMET-GAUDELUS, Dorota SANDS, Gerard LAMBEAU, Philippe MORAND, Michel CHIGNARD*, Yongzheng WU*, Lhousseine TOUQUI*-Unité de Défense Innée et Inflammation, Unité Inserm U. 874, Institut Pasteur, Paris.

Short chain fatty acids (SCFAs) modulate immune response to anaerobic bacteria 02.13.1000.002

Presenter Last Name: **Vinolo**

Previous studies demonstrated that SCFAs, which are products of dietary fiber fermentation by gut microbiota, attenuate inflammation and may be useful in the treatment of conditions such as inflammatory bowel disease and obesity. However, their role in immune response during infectious disease is unknown. We investigated the effect of the main SCFAs (acetate, propionate and butyrate) on macrophage response to the infectious bacteria Aggregatibacter actinomycetemcomitans (Aa), which is implicated in periodontal disease. Thyoglicollate-elicited peritoneal macrophages obtained from C57Bl6 mice and were incubated with Aa or TLRs agonists (LPS or Pam3CSK4) with different non-toxic concentrations of SCFAs. Cytokines (TNF-α, IL-1β, IL-10 and IL-6) and chemokines (Cxcl1 and Cxcl2) were measured in the cell culture supernatant after incubation for 24 hrs. Phagocytosis and killing of bacteria were also evaluated by flow cytometry and plate counting methods. SCFAs modulated cytokine and chemokines production by macrophages: propionate and butyrate reduced the production of TNF-α, NO and IL-6 in response to bacteria and TLR agonists suggesting an anti-inflammatory effect. However, SCFAs inhibited the production of the anti-inflammatory cytokine, IL-10, increased the production of IL-1β by Aa-stimulated cells and the release of chemokines in response to LPS, but not Aa or Pam3CSK4, suggesting that they also present pro-inflammatory actions. The effect of SCFAs on LPS and Pam3CSK4 on cytokines was confirmed in bone marrow-derived macrophages. These effects were independent of IL-10 (the same effect was observed in macrophages from IL-10KO mice), but were in part due to activation of inflammasome since inactivation of caspase-1 abolished some of the effects of SCFAs. Lastly, we also observed that the SCFAs acetate and butyrate reduced phagocytosis and killing of Aa (30% reduction). These results indicate that SCFAs modify important aspects of macrophage effector mechanisms in response to bacteria and that these compounds may affect the outcome of infections.

Eicosapentaenoic and docosahexaenoic acid differentially enhance humoral immunity in murine diet-induced obesity

02.13.1000.003

Presenter Last Name: **Shaikh**

Increased morbidity and mortality in response to infections is a significant and costly complication of obesity. Dietary fat is a critical factor in regulating immune responses and is well studied in relation to cell-mediated immunity and inflammation. In contrast, far less is known about the role of dietary fat on B cell driven humoral immunity, which has a central role in combating infection. Here we show that dietary supplementation with long chain n-3 polyunsaturated fatty acids (PUFA) as either fish oil, eicosapentaenoic (EPA) or docosahexaenoic (DHA) ethyl esters boost B cell activation and antibody production in a murine

model of diet induced obesity. Administration of menhaden fish oil, modeling human pharmacological intake, increased antigen specific antibody production in lean mice and rescued the decrement in IgM antibody levels in obesity. N-3 PUFAs also increased the frequency of select splenic B cell subsets in lean and obese mice. We then determined if EPA and DHA ethyl esters enhanced B cell activity upon supplementation to obesogenic diets. EPA and DHA differentially enhanced B cell activation, with DHA notably increasing B cell IL-10 secretion. EPA and DHA also differentially increased the frequency of B cells, natural IgM and IgA levels. Mechanistic studies in vitro showed the immune enhancing effects of n-3 PUFAs on B cell activation were independent of resolvin D1 and were associated with the ability of EPA and DHA to prevent lipotoxicity induced by palmitate, which is abundant in high fat diets. Taken together, our data establish novel activities for EPA and DHA; that is, enhancement of B cell driven humoral immunity in lean and obese mice.

Effect of omega-3 fatty acids supplementation on expression of NF-kB gene and blood cells integrins in patients with homozygous sickle cell disease 02.13.1000.004

Presenter Last Name: **Daak**

Background: Chronic inflammation and increased adherence of blood cells to vascular endothelium play a critical role in the pathphysiology of sickle cell disease (SCD). There is evidence that omega-3 fatty acids are effective and safe therapy for patients with the disease. However, the mechanisms through which the effect is mediated have not been elucidated. Objective: To investigate the effect of omega 3 fatty acid (DHA and EPA) supplementation on the expression of the pro-inflammatory transcription factor NF-kB and the adhesion molecule integrins in peripheral blood of patients with SCD.
Procedure: Omega 3 fatty acid treated (n=15) and untreated (placebo, n=18) Sudanese homozygous sickle cell patients aged 3 to 18 years and healthy controls (n=22) aged 7 to 18 years were recruited in to the study. The treatment group received 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 two years. The placebo group was given high oleic acid sunflower seed oil capsules. NF-kB gene expression was measured in buffy coat by a real-time qRT-PCR. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference gene. In a sub-sample of the patients, omega-3 treated (n=10) and placebo (n=10), the expression of granulocytes and monocytes integrins were assessed. Results: The expression of NF-kB gene expression in buffy coat was reduced by 33.3% ($p<0.05$) in the omega 3 patients (Relative fold difference (RFD = 0.04) compared with the placebo group (RFD = 0.06). Similarly, the omega 3 group had reduced expression of monocyte integrin ($p<0.05$). There was no difference in granulocyte integrin expression between the two groups

(p>0.05). Conclusion: This exploratory pilot study suggests that the therapeutic effect of omega-3 fatty acid in SCD may be partly due to their anti-inflammatory and antiadhesive actions.

Fatty acid and glycerophospholipid metabolism in human T cells 02.13.1000.005

Presenter Last Name: **Robichaud**

Glycerophospholipid (GPL) and fatty acid (FA) biosynthesis are induced in transformed cells which allow rapid cell proliferation; however, changes in GPL remodeling following cell transformation are not fully characterized. Similarly, little is known regarding these changes following the induction of proliferation of primary non-transformed cells. We measured FA composition of GPL in resting and in proliferating receptor-stimulated primary human T cells. The cellular GPL content was significantly increased in stimulated cells and the FA composition was greatly modified compared to resting cells. Saturated and monounsaturated FA content were significantly increased in proliferating cells as were fatty acid synthase and stearoyl-CoA desaturase-1 gene (9.7±2.1 fold and 169±42 fold, respectively) and protein expression. Additionally, cellular arachidonic acid (AA) was redistributed in cellular GPL classes in a pattern unlike any other FA, with an accumulation in phosphatidylethanolamine (PE) and phosphatidylinositol (PI) species and a loss from phosphatidylcholine (PC) species. In pulse-label experiments, cellular [^{3}H]AA uptake was increased (21.1±1.5 fold) and AA-GPL remodeling, characterized by the transfer of AA from PC to PE, was significantly accelerated in proliferating T cells compared to resting cells. Additionally, arachidonoyl-CoA synthetase, lyso-PC acyltransferase and lyso-PI acyltransferase activities were significantly increased in proliferating T cells. Lyso-PE acyltransferase activity was unchanged. The expression of several phospholipases A_2 (IVA, IVC, VIA, VIB), acyl-CoA synthetases (FATP4, ACSL3, ACSL4, ACSL5 and ACSL6) and lysophospholipid acyltransferases (LPCAT3 and LPIAT1) were also significantly changed as measured by qPCR and western blot. Overall, these results indicate that significance changes in GPL and FA metabolism accompany the induction of cell proliferation and that associated enzymes may be therapeutic targets for the treatment of proliferative disorders.

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Postprandial regulation of Toll-like Receptors (TLRs) by omega-3 fatty acids in obese and normal weight subjects 02.13.1000.006

Presenter Last Name: **Paras-Chavez**

Introduction: Obesity has been associated with low-grade inflammation. Dietary fatty acids may influence inflammation via effects on immune cell function and receptor expression. Toll like receptors (TLRs) are a group of trans membrane proteins participating in the initiation of an immune response. The link between obesity, fatty acids and TLRs has not been well-explored. Objective: To explore the effects of obesity, omega-3 fatty acids (O3FA) and a high fat meal on TLRs expression on monocytes. Methods: This is a double-blind controlled trial. Healthy normal weight and obese adults were recruited. They made 3 clinic visits. A 6 h postprandial test was performed following a high fat meal on each visit. On one of the first two visits the meal included O3FA (acute effect). Between the second and third visit subjects consumed O3FA or placebo for 12 weeks (chronic effect). TLR2 and TLR4 expression on peripheral blood monocytes (CD14+ cells) were determined by flow cytometry. Results: At baseline, normal weight subjects had higher numbers of CD14+TLR2+ cells , higher TLR2 expression, fewer CD14+TLR4+ cells and lower TLR4 expression than obese subjects (all $p<0.05$). The high fat meal caused a transient increase in TLR2 and TLR4 expressing monocytes that was greater in obese subjects ($p<0.05$). Including O3FA with the meal (acute effect) increased TLR2 expression of CD14+ cells from obese subjects ($p<0.05$). Chronic supplementation with O3FA had no significant effect on the postprandial expression of TLRs. Conclusion: The numbers of CD14+TLR2+ and CD14+TLR4+ cells and TLR expression levels differ between normal weight and obese subjects and are affected by a high fat meal, particularly in obese subjects. O3FA alters human immune cell TLR expression when included with a high fat meal. Changes in TLR expression following a high fat meal including O3FA may alter immune cell function and thus inflammatory responses.

Aging changes omega-3 fatty acid homeostasis; implications and challenges 02.14.1000.001

Presenter Last Name: **Cunnane**

Epidemiological studies fairly convincingly suggest that higher intake of fatty fish and omega-3 fatty acids are associated with reduced risk for Alzheimer's disease (AD). Docosahexaenoic acid (DHA) in plasma is normally positively associated with DHA and fish intake so the higher risk of AD in the elderly would be expected to be associated with lower plasma DHA. Despite being associated with lower fish and DHA intake, unexpectedly, plasma (and especially brain) DHA is frequently not lower in AD. Indeed, compared to young adults, DHA is often slightly but significantly higher in plasma and erythrocytes in the elderly in the absence of age-related cognitive decline. Higher plasma DHA in the elderly could be a sign that their fish or DHA intake is higher but we show here that various aspects of DHA homeostasis also change with age. Our supplementation and

carbon-13 tracer studies show that DHA homeostasis, e.g. DHA transit through the plasma (half-life), apparent retroconversion EPA and beta-oxidation, is different in the healthy elderly compared to healthy young adults. Apolipoprotein E4 increases the risk of AD, possibly in part because it too changes DHA homeostasis. Therefore, independent of differences in fish intake, changing DHA homeostasis with aging tends to obscure the relationship between DHA intake and plasma DHA. In turn, changing DHA homeostasis may contribute to making the elderly more susceptible to cognitive decline despite them having similar or sometimes higher plasma DHA than in younger adults. Changing DHA homeostasis in the elderly may help explain why, as a whole, clinical trials with omega-3 fatty acids have not so far been shown to reduce age-associated cognitive decline.

Acknowledgements: CIHR, CRC, CFI, NSERC and FRQS supported for this research.

Telomere shortening in elderly individuals with mild cognitive impairment may be attenuated with n-3 fatty acid supplementation 02.14.1000.002

Presenter Last Name: **Parletta**

Objectives: Excessive shortening of the telomeric ends of chromosomes is a marker of accelerated aging. Oxidative stress and nutritional deficiency may influence this process. The aim of this study was to investigate the effect of omega-3 polyunsaturated fatty acid (n-3 PUFA) supplementation on telomeric shortening in elderly individuals with mild cognitive impairment (MCI). Methods: Thirty-three adults ages > 65 y with MCI were randomized to receive a supplement rich in the long-chain n-3 PUFAs eicosapentaenoic acid (EPA; 1.67 g EPA + 0.16 g docosahexaenoic acid DHA/d; n=12) or DHA (1.55 g DHA + 0.40 g EPA/d; n=12), versus n-6 PUFA linoleic acid (LA; 2.2 g/d; n=9) for 6 months. Results: The intervention did not show an increase in telomere length with treatment and there was a trend toward telomere shortening during the intervention period. Linear mixed modelling produced a robust model although statistically underpowered. Telomere shortening was greatest in the LA group (d =0.21) than in the DHA (d=0.12) and EPA groups (d=0.06). Increased erythrocyte DHA levels were associated with reduced telomere shortening (r=0.67; P=0.02) in the DHA group. Conclusion: Telomeric shortening may be attenuated by u-3 PUFA supplementation, warranting further investigation in larger samples.

Effects of n-3 polyunsaturated fatty acid supplementation on recurrence prevention in patients with late-life depression: a 48-

week randomized double-blind placebo-controlled study
02.14.1000.003

Presenter Last Name: **CHIU**

Background: N-3 polyunsaturated fatty acid (PUFAs) supplementation may be beneficial in depression treatment. Its short-term antidepressant effect in elderly has been supported by an 8-week supplementation study. The aim of this study was to investigate the long-term effects of n-3 PUFA supplementation on recurrence prevention in patients with late-life depression (LLD) by a 48-week randomized double-blind placebo-controlled study Methods: We enrolled 89 patients ≧ 60 year-old with LLD from outpatient psychiatric services of four hospitals. They were randomly assigned to 3 gm n-3 PUFAs or placebo (olive oil) added on their ordinary treatment. Participants received the assessment of demographic data, depression-related variables, and lab exam at baseline. They were followed at week 4, 8, 16, 24, 32, 40, and 48 for assessing recurrence of depression, defined as fulfillment of the criteria of major depressive episode in DSM-4, or suicide subscore of Hamilton Depression Rating Scale ≧3, or hospitalization due to depression during study period. Results: Sixty-four cases, 46 female and 18 male with average age of 66.8±6.4 years, with sufficient data were analyzed. The percentage of previous hospitalization, late-onset LLD (onset of depression ≧60 yrs), and current alcohol drinking in these patients were 33.7%, 44.9%, and 20.3%, respectively. Most adverse effects were tolerable and no group difference was found. The participants in n-3 PUFA group tended to have less recurrence of depression compared to those in placebo group during the 48-week follow-up (27.3% vs. 48.4%; p=0.08). After adjustment for potential confounders by logistic regression model, the difference became significant (Odds Ratio: 0.25, 95% C.I.=0.08-0.84; p=0.025). This difference was more significant in participants with late-onset depression compared to those with early-onset depression. Conclusion: N-3 PUFA supplementation may decrease the recurrence of depressive episodes in older people with LLD. Most of the adverse effects are tolerable and compatible with placebo.

Resolvins in Alzheimer disease patients supplemented with omega-3 fatty acids 02.14.1000.004

Presenter Last Name: **Fiala**

The neuropathology of Alzheimer disease is related to brain amyloidosis with amyloid-beta 1-42. Macrophages, the innate immune cells responsible for amyloid-beta clearance in the brain, are defective in amyloid-beta phagocytosis and degradation in patients with Alzheimer disease. Resolvin D1 (in

concentration dependent fashion 0.26 nM to 260nM) increased amyloid-beta phagocytosis by macrophages of Alzheimer disease patients. In addition, resolvin D1 treatment of peripheral blood mononuclear cells (PBMCs) normalized the transcription of interleukin-1 (IL-1) alpha and beta according to the basal state: down regulated IL-1 in Alzheimer patients with inflammatory PBMCs and up regulated IL-1 in patients with non-inflammatory PBMCs. In addition resolvin D1 differentially down regulated certain chemokines. We are conducting a nutritional study of 10 Alzheimer disease patients receiving supplementation with omega-3 drink (containing 1 gm of DHA and 1 gm of EPA) and are testing macrophages and PBMCs of these patients and controls regarding production of resolvin D1 and transcriptional regulation. The results show that in vivo supplementation with DHA is increasing production of resolvin D1 and modulates transcription of inflammatory genes. Thus, nutritional supplementation with omega-3 has profound biochemical and immune effects possibly related to resolvins in PBMCs of Alzheimer disease patients.

Can DHA enriched Omega 3 fatty acids affect APOE4 -positive patients cognition better in mild to moderate Alzheimer's disease? The Omeg AD study 02.14.1000.005

Presenter Last Name: **Freund-Levi**

Alzheimer (AD) patients with APOE4 negative genes show signs of faster progression rates and prominent cognitive decline. Nutritional studies based on epidemiological, animal and RCT have shown data that an increased intake of DHA enriched Omega 3 fatty acids (n-3FFA) have positive effects on cognition. Material and methods: 174 patients from the OmegAD study were divided into APOE4 - and APOE4 + groups, both received 2.3 gram DHA enriched n-3 FFA. Effects on cognition (using Mini Mental State Examination Test, MMSE, ADAS-CoG), neuropsychiatric symptoms (NPI, MADRS) nutritional biomarkers in plasma n-3 FFAs; EPA, DHA, DPA and n-6 FF, LA AA. Results: 27.3 % were APOE4 -. No significant differences between the APOE+ /- groups was found between age, gender and weight. Between APOE4+ and - groups no significant treatment effects of the supplementation was found in cognitive measures MMSE, ADAS-Cog nor in neuropsychiatric symptoms NPI. However on rates of depressive symptoms-MADRS- a significant difference was observed between the distributions of delta MADRs across APOE4 categories, with decreased levels after 6 months treatment in the APOE4 – group (p -value = 0.006). All patients receiving n-3 FFA supplementation increased in levels of DHA, DPA and EPA. In the APOE4+ group (n=121) the mean value of the n-3FFA delta EPA was higher (mean=3.8 ±1.4) as compared to the APOE- group (n=47, mean=3.3 ±1.5). There was a significant difference (p=0.026) between the distributions of dEPA across the APOE4 groups. No effects on DHA and DPA levels was found. No treatment effects was measured in the APOE4+ /- group in

the n-6 FFAs albeit decreased ns levels of dLA and dAA. Conclusion: For APOE4+ Alzheimer patients there was a clear treatment benefit of 6 months supplementation with n-3 FFAs for depressive symptoms and higher values of plasma dEPA as compared to APOE4-patients was found

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Effects of a whole diet intervention and FADS2 genotype on fatty acid status in the elderly 02.14.1000.006

Presenter Last Name: **O'Neill**

The proportion of people >65y in Europe is predicted to increase from 25 to 40% by 2030 [1]. It is important to identify dietary strategies that will contribute to healthy ageing. NU-AGE is a 5 centre trial involving 1,250 adults (65-79y) with the aim of examining the impact of a dietary intervention (including advice on Eicosapentaenoic acid and Docosahexaenoic acid) on chronic low grade inflammation and cardiovascular health. The effects of intervention on plasma fatty acid status and vascular function will be investigated in Norwich participants. Vascular function is measured using Pulse Wave Velocity (PWV), Cardio-Ankle Vascular Index (CAVI) and EndoPAT. Table 1 displays some of the characteristics of this population at baseline. Table 1: Characteristics of the Nu-Age cohort (n=154) at baseline Characteristic Mean (SD) Age (years) 69.9 (4.0) BMI (kg/m2) 27 (5) Systolic Blood Pressure (mmHg) 139 (18) Diastolic Blood Pressure (mmHg) 77 (9) PWV (m/s) 9.2 (1.8) CAVI 8.8 (1.2) Reactive Hyperaemic Index (RHI) 2.5 (0.8) The vascular health of the Nu-Age population is slightly above average; a PWV of 9.2 is healthier than aged-matched groups [2], with mean CAVI results similar to other studies for this age group [3] and 10.4% of the Nu-Age population have endothelial dysfunction. Plasma fatty acid and vascular function results before and after intervention will be presented at the conference. The impact of individual fatty acid desaturase (FADS2) gene variants and haplotypes on plasma fatty acids will be discussed. The FADS2 enzyme is responsible for the desaturation of fatty acids in the synthesis of EPA from α-linolenic acid. 1. UN, World Population Ageing: 1950-2050, 2002. 2. T.R.V.f.A.S., Determinants of PWV in healthy people & in the presence of CV risk factors. 2010. 3. Namekata, et al., Establishing baseline criteria of CAVI as a new indicator of arteriosclerosis: a cross-sectional study. 2011

Maternal DHA Supplementation during Pregnancy and Body Composition in Childhood: Results of the 3 and 5 Year Follow-up of Children Born to Women in a Randomized Controlled Trial of DHA Supplementation during Pregnancy 02.15.1000.001

Presenter Last Name: **Muhlhausler**

Background: Previous studies have suggested that exposure to an increased supply of n-3 LCPUFA before birth may result in persistent reductions in fat mass, however current evidence is inconclusive. Objective: To determine the effect of high-dose maternal DHA supplementation on children's body mass index (BMI) z-score and body fat mass at 3 and 5 years of age. Method: We conducted a follow-up of children born to mothers enrolled in the DOMInO (DHA to Optimize Mother Infant Outcome) RCT between March 2009 and October 2013. Pregnant women were supplemented with DHA-rich fish oil capsules (800 mg DHA/d) or vegetable oil capsules (control group) from 20 weeks gestation until birth. Weight, height, waist and hip circumferences were recorded at 3 and 5 years of age and BMI z-score calculated. Body fat mass was assessed using bioelectrical impedance. Fasting blood samples were collected at 5 years of age for determination of HOMA-IR. Diet and physical activity data were obtained by parental questionnaire. The primary outcome was BMI z-score at 3 and 5 years of age. Results: Of the 1660 children eligible, 1502 (92%) completed assessments at 3 years and 1407 (85%) completed assessments at 5 years. Preliminary analyses of the 3-year data showed no significant difference in the BMI z-score between the Control and DHA groups (unadjusted mean difference 0.03, 95% CI -0.08-0.13, P=0.60). Body weight, height, waist and hip circumferences at 3 years were also not different between groups. Data collection for the 5 year assessments has been completed, however the study is still blinded and analysis underway. Data for the full study will be presented at the meeting. Conclusions: Increasing prenatal DHA exposure does not appear to affect BMI z-score at 3 years of age, however results of the full analysis are required before any conclusions can be drawn.

Fish Oils Supplementation During Pregnancy and Child Neurodevelopment 02.15.1000.002

Presenter Last Name: **Bjarnadóttir**

Background: A number of randomized controlled trials have investigated a potential causal relationship between docosahexaenoic acid (DHA) intake during pregnancy and neurodevelopment in the children, but the evidence is not conclusive. Objective: The Copenhagen Prospective Study on Asthma in Childhood (COPSAC2010) aim to examine among other the effect of fish oil during pregnancy on neurodevelopment during early childhood. Methods: A total of 738 women were randomized 1:1 to a daily supplement of fish oil (2.4 g/day of n-3 fatty acid; 55% eicosapentaenoic (EPA) and 37% DHA) or matching olive oil from pregnancy week 24 to 1 week after delivery. The main neurodevelopmental outcome in the trial is cognitive-score on the Bayley Scales of Infant and Toddler Development (third-edition, BSID-III) measured at 30

months of age. Secondary outcomes are developmental milestones monitored prospectively by parents using a registration form based on the Denver Developmental Index and WHO milestones as well as language development at 12 and 24 months using the MacArthur Communicative Development Inventory (CDI) for infants and toddlers, respectively. Results: After birth, 698 neonates were enrolled. Mean gestational age was 39.9±1.7 weeks, and mean birth-weight 3.5±0.5 kg. Thirty-seven children were excluded post-randomization. Neurodevelopmental outcomes have been assessed in 661 children of whom 91% completed the BSID-III test, 92% had information on ≥1 milestones, and 49% and 72% had complete CDIs at 12 and 24 months, respectively. The 3-year follow-up will be completed in March 2014 and the trial will then be unblinded, allowing presentation of new unpublished data at the ISSFAL meeting. Flow chart of the study participants through the Trial and main outcome measures

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Gender differences in associations between dietary fatty acids and blood lipids: the PURE study South Africa 02.15.1000.003

Presenter Last Name: **Richter**

Background: A higher prevalence of dyslipidemia than previously documented in South African studies was recently seen in preliminary data from the Prospective Rural Urban Epidemiology (PURE) study. Individual dietary fatty acids may have a different effect on blood lipids than groups of fat (i.e. saturated fatty acids). Therefore, we investigated the dietary intake of individual fatty acids and their associations with blood lipids, taking into account urbanization and gender. Methods: Cross-sectional data analysis within the PURE baseline study of healthy subjects (n = 1950, 35–70 years) from rural and urban areas. Dietary data were collected by means of interviewer-based quantitative food frequency questionnaires and blood lipid analysis performed. Results: Dietary fat intake was higher among urban dwellers than rural ones and individual fatty acids followed the same trend. Total cholesterol and LDL were higher in females than males, with no differences between rural and urban dwellers. Intake of alpha-linolenic acid (ALA) was positively associated with total cholesterol ($\beta = 0.143$, $p = 0.038$) and triglycerides ($\beta = 0.256$, $p < 0.001$) in males. The risk for having elevated LDL increased with increased intake of ALA (OR 1.49, 95% CI 1.04, 2.14) in males. In females, arachidonic acid ($\beta = 0.113$, $p = 0.014$) and eicosapentaenoic acid ($\beta = 0.260$, $p = 0.023$) were positively associated with total cholesterol and arachidonic acid was also positively associated with LDL, whereas docosahexaenoic acid was negatively associated with total cholesterol and LDL ($\beta = -0.243$, $p = 0.037$). Conclusions: Individual dietary fatty acids may have different effects on blood lipids of males than females, regardless of urbanization. The positive association between ALA and total cholesterol and triglycerides in males is of interest and concern, but might be explained by further biomarker analyses.

CONSUMPTION OF BIOACTIVE MOLECULES FROM HUMAN MILK AND RELATIONSHIP TO INTESTINAL MATURITY IN PREMATURE NEONATES 02.15.1000.004

Presenter Last Name: **Armand**

Background: Human milk provides numerous bioactive molecules contributing to optimal development of neonates. Among them, phospholipids (such as sphingomyelin, SM), LC-PUFA (AA, DHA) and proteins (SMase, sCD14) might be involved in intestinal maturity. Objectives: We aimed to quantitate their real intakes in an early postnatal critical window (first month of enteral nutrition) and to determine whether they could be related to intestinal maturity. Procedure: Sixty-six neonates (1253±306 g, 29.6±1.3 wks) were followed up from the start of minimal enteral feeding with own mother's milk (MM, n=23) or pasteurized milk from donors (DM, n=43) until 4 weeks. Representative samples were analysed by GC for fatty acid composition, by 31P NMR for phospholipids class quantification. Acid SMase activity was measured using radiolabelled SM and sCD14 by ELISA test. Intestinal maturity was defined as the number of days for reaching total enteral feeding. Data are means ± SD or mean ranges over 4 weeks. Statistics were performed using SPSS ($p < 0.05$). Results: The consumption of DHA (41-139 mg/wk) was inferior to recommendations (40-70 mg/d) due to moderate levels in milk (0.34± 0.10%) and to low administrated volumes (4.0±1.5 or 3.8±1.5 L/month for MM or DM). The intake of SM (19-57 mg/wk) was inferior to amounts shown beneficial for intestinal development (60-150 mg/d). Acid SMase activity was higher in MM vs DM (308 ± 77 vs 201 ± 78 pmol/h/ml). The intake of sCD14 was higher with MM vs DM (2.2-8.5 vs 0.4-4 mg/wk). Negative correlations were found between the ingested amount of specific phospholipids and age of full enteral feeding (EPLAS, PC, SM, PS), time to reach full enteral feedings (EPLAS, PI, SMase), digestive disorders (EPLAS, PI, PS), or infections (EPLAS). Conclusions: SMase and specific phospholipids from human milk appear as important nutrients for neonatal gut development, a higher consumption being beneficial.

Effect of maternal DHA supplementation on body composition of 5-year-old children 02.15.1000.005

Presenter Last Name: **Vetri Villalan**

Background: Obesity is a worldwide problem that often begins with excess fat accumulation in early childhood. Recent observational studies have linked childhood body fat to maternal n-6 and n-3 PUFA status during pregnancy. Objective: To compare % body fat to BMI %ile in children whose

mothers were randomly assigned to 600 mg/day DHA (n=37) or placebo (n=33) before 20 weeks gestation until birth. Methods: In the first 70 children enrolled in an ongoing study of the effects of prenatal DHA on childhood body composition, we assessed fat mass (FM) and fat free mass (FFM) by air displacement plethysmography (BODPOD*) and compared BMI %ile (http://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/tool_for_schools.html) to % body fat and % FFM for each group at 5 years of age. Several regression models were performed and the model with unequal slopes was chosen for final analysis. Results: In the placebo group, % body fat increased and % FFM decreased by 0.15% for every 1% increase in BMI %ile ($p<0.001$). In the DHA group the changes in % body fat and % FFM were not significant ($p=0.1008$). Conclusion: Intrauterine exposure to DHA may program children to accumulate different proportions of fat and lean mass as BMI increases.

Nervonic acid in early plasma samples from premature infants correlates with birth size and mental and motor development up to 18 months corrected age. 02.15.1000.006

Presenter Last Name: **Ntoumani**

Objectives: Nervonic acid (NA) is associated to white matter volume and is a major component of myelin. The objective of this prospective observational study was to investigate the association of early NA concentration with premature infant development up to 18 months corrected age. Material and methods: The fatty acid pattern in early plasma phospholipids and breast milk of 51 premature infants have previously been reported (Lipids Health Dis 2009; 8:20). Developmental parameters at 6, 10 and 18 months were investigated with Bayley´s Scale of Infant Development II. Mann Whitney´s U-test and multiple regression analyses adjusted for other fatty acids and confounders were used. Results: The NA concentration in plasma phospholipids at 1 week correlated to birth weight SDS ($r=0.46$ $p=0.003$) and head circumference SDS ($r=0.44$ $p=0.006$) and was lower in SGA than AGA infants ($p=0.003$). The NA concentrations of plasma phospholipids at 1 month correlated significantly with developmental outcome in Bayley´s motor scales at 6 months ($r=0.35$, $p=0.022$), mental and motor development at 10 months ($r= 0.36$, $p=0.017$ and $r=0.39$, $p=0.007$, resp.), as well as orientation and emotional development ($r=0.37$, $p=0.014$ and $r=0.35$, $p=0.019$, resp.); and with mental development at 18 months ($r=0.30$, $p=0.046$). In multiple regression NA concentration in plasma phospholipids at 1 month was significantly associated with mental development (β 0.28, R^2 0.25), and motor development (β 0.32, R^2 0.29) at 10 months. No correlations were found to breast milk NA concentration at 1 week even though milk from mothers of preterm infants had sevenfold increased concentrations of NA compared to milk of mothers of term infants ($p=0.0003$). Conclusion: The results suggest that NA is positively associated to birth weight and head

circumference in preterm infants. NA in plasma at 1 month was positively associated with developmental outcome at 10 months. Follow-up to 5 yrs is in progress.

Oxysterols and the brain 02.16.1430.001

Presenter Last Name: **Bjorkhem**

An immense knowledge has accumulated concerning regulation of cholesterol homeostasis in the body. This does not include the brain, however, in which detailed mechanisms are just emerging. Approximately 25% of the cholesterol present in the human body is localized to this organ. Almost all brain cholesterol is a product of local synthesis with the efficient blood-brain barrier protecting it from exchange with lipoprotein-bound cholesterol in the circulation. Almost 20 years ago we showed that the most important mechanism for elimination of cholesterol from the brain involves conversion into a metabolite that is able to pass the blood-brain barrier, namely 24S-hydroxycholesterol. The activity of the enzyme responsible for the 24S-hydroxylation (CYP46) is of importance for cognition. Knock out or overexpression of this enzyme leads to reduced and increased spatial memory function respectively in mice. This flux also reduces the generation of β-amyloid in the brain We have shown that another side-chain oxidized oxysterol, 27-hydroxycholesterol, fluxes from the circulation into the brain. This flux has a negative effect on cognition and we have shown that at least part of this effect is mediated by the “memory protein” Arc. The two enzymes responsible for the flux of the two oxysterols across the blood-brain barrier in opposite directions are promising drug targets in connection with neurodegeneration.

A Novel Role for Very Long Chain Fatty Acids in Brain Function 02.16.1430.002

Presenter Last Name: **Hopiavuori**

Purpose: ELOngation of Very Long chain fatty acids-4 (ELOVL4) is an elongase responsible for biosynthesis of very long chain (VLC; ≥C28) fatty acids found as components of more complex lipid molecules such as sphingolipids and phosphatidylcholine. ELOVL4 synthesizes the VLC polyunsaturated fatty acids (VLC-PUFA) in retina and testes, and VLC saturated fatty acids (VLC-FA) in skin and brain. Heterozygous inheritance of mutant ELOVL4 causes juvenile macular degeneration in autosomal dominant Stargardt’s macular dystrophy (STGD3). A 2011 case study reported that homozygous inheritance of the STGD3 mutation causes a central nervous system (CNS) phenotype in humans, including seizures, intellectual disability, spastic quadriplegia and death. We hypothesize that

ELOVL4-synthesized VLC-FA play an essential role in neural cell structure and function. Methods: We generated the first successful animal model for STGD3/STGD3 inheritance by also expressing wild-type ELOVL4 in skin. Wild-type ELOVL4 localization within the CNS was determined using immunofluorescence. Brain lipids were extracted from wild-type hippocampi and separated into classes by solid phase extraction (SPE) before GC/MS analysis. Positron emission tomography (PET) was used to assess CNS uptake of fluorodeoxyglucose (FDG) in STGD3/STGD3 mice. Results: Our STGD3/STGD3 mice recapitulate the human phenotype, developing running seizures at P19 followed by death at P21. ELOVL4 has known expression in brain, but the specific pattern was undescribed. We found the highest enzyme immunoreactivity within the hippocampus, in neurons (not glia) of the sub-granular layer of the dentate gyrus (DG). GC/MS following SPE confirmed the presence of 28:0 and 30:0 in sphingolipids. PET imaging of STGD3/STGD3 mice revealed a 3-fold increase in the amount of FDG uptake into the CNS. Conclusions: This is the first study to demonstrate mutations in Elovl4 causing a CNS phenotype in an animal model, implicating for the first time a potential role of VLC-FA in neural cell structure and function.

Full hydrogenation suppresses life-span shortening activity of canola oil in SHRSP 02.16.1430.003

Presenter Last Name: **Tatematsu**

Several vegetable oils including canola oil (Can) shorten the life span of stroke-prone spontaneously hypertensive rat (SHRSP). Similar effect was also demonstrated in partially hydrogenated Can and soybean oil, but the action of fully hydrogenated Can on the life span has not yet been examined. To clarify the impact of full hydrogenation, we compared the life span of SHRSP fed the diet containing fully hydrogenated Can (FHCO) with that of the animals given other oils under 1% NaCl loading. A basal diet for rodents and one of the test oils, FHCO, Can, Lard (Lrd; control oil which does not induce life-span shortening) or palm oil (Plm; having similar melting point to Lrd), were mixed at a 9:1 ratio, and the diets were given to male SHRSP after weaning. Fatty acid composition of Can diet was 52.9% oleic acid (18:1), 26.1% linoleic acid (18:2n-6) and 7.2% alpha-linolenic acid (18:3n-3). FHCO diet contained more than 60% stearic acid (18:0) and no trans fatty acid was detected. Lrd and Plm had a similar ratio of palmitic acid (16:0), oleic acid and linoleic acid. In early term, an increase in food ingestion (15-20%) was observed in SHRSPs fed FHCO compared with other dietary groups. However, the body weight gain was decreased significantly in Can and FHCO, compared with other two groups at 11-week old. The survival times of SHRSPs were in order of Can (94±3 day), Plm (101±2 day) and Lrd (115±6 day), and rats fed (with) FHCO diet were not died during the experimental period (180 < day). These results suggest that full

hydrogenation of Can converts possible causatives of life-span shortening to some safety substance or poorly-absorbable forms. Furthermore, this reaction might provide preventive substances for stroke.

Omega-3 Fatty Acids (Omegaven) protect from Mitochondrial Dysfunction in a MCAO mouse model of stroke 02.16.1430.004

Presenter Last Name: **Eckert**

Recent investigations demonstrated efficacy of docosahexaenoic acid (DHA) to reduce stroke size and severity in the transient middle cerebral arterial occlusion (MCAO) model in rats when applied intravenously after reperfusion. In this study we investigated the beneficial effect of OMEGAVEN (Fresenius Kabi, Germany) a medical lipid emulsion for parenteral nutrition that contains the long-chain omega-3 fatty acids eicosapentaenic acid (EPA) and DHA in a model of transient stroke. Mice underwent transient MCAO and OMEGAVEN was administered intravenously (5 ml/kg b.w.) after stroke (90 min) at reperfusion that represents an early moment for potential intervention. The degree of damage, mitochondrial function and neuroinflammation were investigated. Treatment with OMEGAVEN significantly decreased the stroke area by 21% and lowered the severity of stroke by 50%. OMEGAVEN significantly improved mitochondrial membrane potential (MMP) and ATP levels in the ischemic brain hemisphere. These findings are accompanied by an enhanced mitochondrial function, e.g. improved respiration of the complexes responsible for oxidative phosphorylation in mitochondria isolated from the ischemic brain hemisphere. The inflammation markers COX-2 and iNOS significantly decreased after treatment with OMEGAVEN. This pilot study demonstrated that OMEGAVEN could represent a promising, approved lipid emulsion for the early therapeutic intervention in ischemic stroke.

THE INFLUENCE OF A SINGLE NUCLEOTIDE POLYMORPHISM IN THE CYP4F2 GENE ON PLATELET EPOXYEICOSATRIENOIC ACIDS AND PLATELET AGGREGATION. 02.16.1430.005

Presenter Last Name: **Barden**

Background: Cytochrome P450 metabolites of arachidonic acid are increasingly being recognized as important modulators of vascular function. Epoxyeicosatrienoic acids (EETs) are generated by epoxygenases and are vasodilators, anti-aggregatory and profibrinolytic. The physiological actions of EETs are limited by metabolism to dihydroxyeicosatrienoic acids (DHETs) by soluble epoxide hydrolase. A guanine-to-adenine polymorphism at position 1347 in the CYP4F2 gene has been associated with hypertension, stroke and elevated

20-hydroxyeicosatetraenoic acid excretion but its effects on CYP450 epoxygenase products (EETs) are not known. Aim: To examined the effect of the G/A 1347 CYP4F2 polymorphism on platelet EETs and platelet aggregation in overweight volunteers. Methods: Fifteen volunteers who were carriers of the A-allele (GA or AA) and 12 volunteers without the polymorphism GG genotype were studied. Platelet aggregation studies were performed in platelet rich plasma in response to collagen (1mg/ml). Platelet EETs and DHETs were measured before and after stimulation with collagen by gas chromatography mass spectrometry. Results: Platelet aggregation to collagen in volunteers with the CYP4F2 GA/AA genotype was significantly greater (75.8%, CI 74%, 77.7%) than that of the CYP4F2 GG genotype (n=12), (68.7%, CI 67.9%, 69.5%) ($p<0.0001$). Collagen stimulation associated with lower platelet EETs levels in the CYP4F2 GA/AA genotype compared with the CYP4F2 GG genotype ($p=0.037$). The lower platelet EETs in the A-allele carriers was not associated with increased metabolism to DHETS. Conclusion: These results suggest that the CYP4F2 polymorphism may be an important determinant of platelet hyper-reactivity affecting the ability of platelets to increase their EET levels in response to a thrombotic event.

Fatty acids and the child's eye 02.17.1430.001

Presenter Last Name: **Hellstrom**

Most fatty acids, important for development and especially the omega-3 polyunsaturated fatty acids (PUFA) for the eye and brain development are transferred in the third trimester. This means that in prematurely born infant as the placental transferal is interrupted the infant is dependent on the concentrations in breast milk, which varies based on the mother’s diet and storage. It has even been suggested that low omega-3 PUFAs would be a cause of premature delivery. Many countries have much higher levels of omega3 fatty acids in breast milk than found in Sweden and breast milk substitutions are generally now supplemented with the LCPUFA. The role of lipids in the development of Retinopathy of prematurity (ROP) is not well defined. We hypothesized that because premature infants today mainly receive omega-6 PUFAs as part of total parenteral nutrition and almost no omega-3 PUFAs, lack of omega-3, PUFAs might contribute to the development of ROP. The retina is normally rich in omega-3 PUFAs. Moreover, the retinal tissue status of certain long-chain PUFAs is dependent on and modified by dietary intake, making lipids an attractive potential intervention for disease. The omega-6 PUFA arachidonic acid (AA, C20:4n-6) is the substrate for pro-inflammatory eiconasanoids generated via the cyclooxygenase (COX) and lipoxygenase (LOX) pathways. COX and LOX are both inducible and constitutively expressed in the retina. The omega-3 PUFA eicosaenoic acid (EPA) is the substrate for anti-inflammatory or substantially less inflammatory eicosanoids and suppresses the production of pro-inflammatory eicosanoids. EPA operates with the omega-3 PUFA

docosahexaenoic acid (DHA) to competitively inhibit omega-6 PUFA biosynthesis by quenching elongation, desaturation, and oxidation enzymes to decrease membrane AA. When we fed a mouse model a diet rich in either omega-3 PUFAs or omega-6 PUFAs, we found that mice on the omega-3 diet had less initial vessel loss and nearly 50% less pathological vessel growth. An effect on dampening inflammation was also implicated, as the retinas of mice recieving the the omega-3 PUFA-rich diet exhibited decreased production of tumor necrosis factor-alpha (TNF-α), a key inflammatory mediator, whereas TNF-α levels increased in the retinas of mice on the omega-6-rich diet. We are now attempting to identify another omega-3 active metabolites and mediators who correlate with ROP development in the mouse. This information will be translated to the clinic by correlating the serum levels of these metabolites with ROP. Based on our experimental and clinical findings, a randomized, prospective, controlled clinical study is being performed at The Queen Silvia Children's hospital in which sufficient doses of omega-3 PUFAs are administered to infants to evaluate their preventive effects on ROP and other preterm morbidity. Preliminary data will be presented. In addition, we have performed longitudinal studies in preterm infants analyzing the adipocytokines adiponectin and leptin, and these results will be presented in relation to growth and metabolism.

Dietary omega 3 long chain polyunsaturated fatty acids and metabolic syndrome in the rat retina: consequences on retinal functionality and complications 02.17.1430.002

Presenter Last Name: **Thierry**

Metabolic syndrome (MetS) is a major risk factor for type 2 diabetes (T2D). Diabetic retinopathy, that encompasses neovascularization and edema, is a prominent complication of T2D that concerns 30-40% of T2D patients. Omega-3 long chain polyunsaturated fatty acids (LC-PUFA) have been associated with the prevention of MetS. The purpose of our study was first to define whether MetS offers favorable conditions for the development of retinal complications. Secondly, we aimed to evaluate if dietary omega-3 LC-PUFA efficiently protected the retina from MetS-associated retinal function impairments and complications. MetS was induced by feeding rats with a 60%-rich fructose diet for up to 3 months. Standard and Fructose diets contained similar equilibrated levels of linoleic and alpha-linolenic acids. Omega-3 supplemented diets contained 10.9% EPA and 7.2% DHA (% of total fatty acids) (n=16 rats in each group). MetS was exemplified by measurements of plasma glucose, lipids, leptin and insulin, adiposity by NMR, and liver steatosis. Choroidal neovascularization was induced by laser argon burns in the fundi, and followed by confocal scanning laser angiography. Retinal functionality was recorded by scotopic electroretinography. Omega-3 LC-PUFA amplified plasma insulin increase induced by fructose but restored leptin levels. We reported a significant loss of cone sensitivity after 8

days of fructose which was not prevented by omega 3s. Adiposity was substantially raised after 3 months in rats fed with fructose diet and exacerbated by omega-3s. Choroidal neovascularization was significantly increased in Fructose diets at 1 month (+54% compared to standard) and but not prevented by omega-3s. Unexpectedly, omega-3 induced localized atrophy of the retinal pigment epithelium and peripheral ischemia in the laser-treated eyes. MetS was characterized by partial loss of cone sensitivity and exacerbated choroidal neovascularisation, and offers favorable conditions for the development of retinal complications. Dietary omega-3 LC-PUFA worsened MetS-associated retinal complications.

Dietary fatty acids and the prevention of Age-related Macular Degeneration: retinal incorporation and beyond 02.17.1430.003

Presenter Last Name: **Bretillon**

Age-related Macular Degeneration (AMD) is the leading cause of visual impairment after the age of 50 years in Western populations. Observational epidemiology reports the prevention of AMD by low linoleic acid intake and the consumption of omega 3 long chain fatty acids. Nevertheless, the mechanisms behind this association are partly elusive. In particular, the relative contribution of circulating fatty acids and in situ metabolism to the fatty acid profile of the retina is unknown. As contributors to the lipid core of lipoproteins, Cholesteryl Esters (CEs) are the prominent carriers of fatty acids in a non post-prandial state in humans. Lecithin Cholesterol Acyl Transferase (LCAT) is the main lipid transfer protein which activity is involved in the fatty acid profile of CEs in LDL and HDL. Our recent findings suggest that CEs contribute to the uptake of circulating omega 3 fatty acids by the retina. To get insights into the flux of fatty acids between plasma compartments and the retina, we took advantage to the analyses of human samples collected from nine donors: retina, retinal pigment epithelium+choroid (RPE/Ch), adipose tissue, plasma lipids, and erythrocytes. The higher linoleic acid in plasma CEs, phosphatidylcholine (PC) and adipose tissue, the greater linoleic acid in RPE/Ch ($r=0.96$, 0.94 and 0.87, $p<0.001$, respectively). Linoleic acid in plasma CEs also mirrored linoleic acid in the retina ($r=0.65$, $p=0.05$). Docosahexaenoic acid in RPE/Ch and LCAT activity (plasma CE/PC) were in association with each others ($r=0.67$, $p<0.05$). Similarly, linoleic acid in the retina was closely associated with circulating LCAT activity ($r=0.73$, $p=0.03$). Fatty acid profile of circulating CEs and LCAT activity are potent determinants for fatty acid composition of the human retina. Beyond plasma fatty acids, remodeling of CEs and lipoproteins may be of peculiar importance in the prevention of AMD by a dietary approach. .

DNA Sequence Variation in Lipid-Associated Signaling Pathway

Constituents, Drug Targets, and Age-Related Macular Degeneration 02.17.1430.004

Presenter Last Name: **SanGiovanni**

Background. Age-related macular degeneration (AMD) is the primary cause of vision loss in elderly people of European ancestry. Biologic plausibility of LCPUFA-retinal disease relationships is supported by: 1) intake-dependent and -modifiable accretion of LCPUFAs to the retina; 2) preferential concentration and localization of LCPUFAs in healthy retinal cells of types manifesting retinal pathology in AMD; and 3) biophysical and biochemical capacity of LCPUFAs to affect processes implicated in AMD pathogenesis. Cholesterol metabolites contribute to chronic inflammatory processes implicated in AMD pathogenesis and progression. Methods. We analyzed findings from large-scale genotyping projects on the molecular genetics of AMD (17,181 people with AMD + 60,074 elderly AMD-free controls) with data from the 1000 Genomes Project, ENCODE, LIPIDMAPS, and pharmacogenomics databases to identify AMD-associated DNA sequence variants resident in genes encoding proteins involved in lipid synthesis, capture, metabolism, and transport. Public-access gene sets were used with pathway analysis software to make inferences in the context of biochemical systems. P-value for association: 5.0E-3. Results. Our findings confirm presence of neovascular AMD-associated DNA sequence variants identified in humans using systems-based analyses on PPAR-RXR and Akt/PI3K signaling constituents. PPAR-RXR pathway genes carrying AMD-associated SNPs included PPARGC1A (rs13106578), NCOA2 (rs17676138), PPARD (rs6902123), and ESRRG (rs1339357). Akt/PI3K pathway genes carrying AMD-associated SNPs included PLCG2 (rs11640294), PIGK (rs1048575), PIK3R1 (rs173702), ITPR2 (rs11048506), INPP5A (rs913196), PIP5K1B (rs3812537). Our findings also provide the first human evidence supporting the role of LCPUFA-related influence of ALOX5 (rs7077173) on pathologic retinal angiogenesis and elucidate a drugable AMD-associated SNP in CETP (rs5882) with the capacity to change protein structure in an evolutionarily conserved domain responsible for binding acyl chains of lipopolysaccharides and neutralizing these molecules on outer membranes of Gram-negative bacteria. Conclusions. Gene products of PPAR-RXR and Akt/PI3K signaling system constituents, ALOX5, and CETP are: 1) influenced by lipid intake; and, 2) associated with AMD.

Molecular Principles for Retinal Pigment Epithelial Cell/Photoreceptor Survival Targeting the NALP3 Inflammasome by Lipid Mediators 02.17.1430.005

Presenter Last Name: **Bazan**

Age-related macular degeneration (AMD) encompasses damage and survival failure of retinal pigment epithelial (RPE) cells, leading to photoreceptor cell death and blindness. A specific RPE cell damaging event is involved in the decrease of the ribonuclease (RNase) DICER1 that, in turn, results in increased abundance of repetitive, mobile, retrotransposon, non-coding cytotoxic Alu RNAs, which have been identified in geographic atrophy (GA), an AMD form that causes blindness in millions of individuals (Kaneko et al., Nature, 2011;Tarallo et al., Cell, 2012). Alu RNA-induced RPE cytotoxicity targets the inflammasome, which participates in a response of the innate immune system to infection or injury by inducing a signaling cascade that results in synthesis of pro-inflammatory mediators, including interleukin 1β (IL-1β) and IL-1α. Alu RNA activates the adaptor protein ASC-PYCARD by bringing together the NOD-like receptors NLRP3 and pro-caspace-1, a protease that, in turn, generates mature caspase-1 to cleave IL-1β and IL-18. We have uncovered that the lipid mediator neuroprotectin D1 (NPD1; 10R,17S-dihydroxy-docosa-4Z,7Z,11E,15E,19Z hexaenoic acid), derived from docosahexaenoic acid (DHA), counteracts Alu-RNA-mediated NALP3 inflammasome activation and that, in turn, potently enhances RPE cell survival. Thus, our results show that either Alu-RNA accumulation or H2O2/tumor necrosis factor α (TNF-α)-induced cell death was down regulated by NPD1, DHA plus the neurotrophin pigment epithelium derived factor (PEDF), or by Glyben, an inflammasome inhibitor. DHA plus PEDF facilitate the endogenous synthesis of NPD1. The DHA-derived mediator, NPD1, is made on demand in the RPE cell and in other cells at the onset of uncompensated oxidative stress conditions and aims to counteract consequences of proteostasis dysfunctions and excessive neuroinflammation, and to restore homeostasis. Moreover, our results indicate that NPD1 or DHA plus PEDF modulate NLP3 transcription and, in turn, the formation of the NLP3 protein that leads to its assembly along with ASC-pycard and procaspase 1, which converts procaspase-1 to mature caspase-1 and, in turn, generates IL-1β and IL-18. Thus, our results identify a specific endogenous lipid–mediated, cell-survival signaling mechanism that counteregulates Alu-RNA induction of the NALP3 inflammasome, resulting in RPE cell survival and essential for photoreceptor cell functional integrity. (Ernest C. and Yvette C. Villere Endowed Chair of Retinal Degenerations, Eye, Ear Nose & Throat Foundation, National Eye Institute grant EY005121)

Fatty acid metabolism in obesity 02.18.1430.001

Presenter Last Name: **Vidal-Puig**

Awaiting final submittal

Association between Metabolic Syndrome and Erythrocyte Fatty

Acid Profile in Mexican Adolescents: A Trans Fatty Acid Approach 02.18.1430.002

Presenter Last Name: **Maldonado-Hernández**

The type of fat consumed in the Mexican diet could predispose to the development of Metabolic Syndrome (MS) which has been associated with an increased risk to develop cardiovascular disease and type 2 diabetes mellitus. Our study included adolescents between 12 and 16 years of age, divided in two groups: Control Group (n=31) and MS Group (n=44). Waist circumference, blood pressure, fasting glucose, triglycerides, and HDL-cholesterol were determined. Erythrocytes' fatty acids methyl esthers were quantified using gas chromatography with ionized flame detector. We identified 16 fatty acids (FA) with chain lengths from C12 to C24, with emphasis in four trans FA (TFA) isomers: vaccenic (C18:1n7t), elaidic (C18:1n9t), linoelaidic (C18:2n6t), and conjugated linoelaidic acids (C18:2n7t). MS Group had a less proportion of: myristic (C14), palmitoleic (C16:1), C18:1n7t, and linoleic acids (C18:2); and a higher one of C18:1n9t, C18:2n7t, and nervonic acids (C24:1) when compared to the control group. C24:1 and C18:1n9t had a significant positive association with MS (OR=14.17 and OR=12.94, respectively); whereas C14 (OR=0.14), C18:1n7t (OR=0.14), and C18:2 (OR=0.22) appear to have a protective effect against the disease. The proportion of specific FAs in erythrocytes' membranes differs between adolescents with MS and healthy controls; these FA not only showed a strong association with MS, but also correlated with most of its individual components. Interestingly, TFA displayed an antagonic behavior; while C18:1n9t had a strong association with MS, apparently C18:1n7t confers a protective effect; these results suggest that analyzing each TFA separately will constitute a more accurate approach to determine the role of TFAs in the pathogenesis of MS or other related metabolic disorders.

Benefits of purified long chain omega-3 fatty acids in non-alcoholic fatty liver disease (NAFLD): Results from the WELCOME study 02.18.1430.003

Presenter Last Name: **Calder**

Non-alcoholic fatty liver disease (NAFLD) is common and increases risk of type-2 diabetes and cardiovascular disease. In a randomised, double-blind, placebo-controlled trial we tested, in 103 patients with NAFLD, whether 15-18 months

treatment with Omacor (4 g/day) [containing EPA and DHA as ethyl esters] increased EPA and DHA in erythrocytes, decreased liver fat, improved two validated biomarker scores for liver fibrosis, and improved cytokeratin 18 (CK-18), a marker of non-alcoholic steatohepatitis. Ninety-five patients completed the study and compliance, based upon returned capsule counting, was high. Mean baseline erythrocyte EPA and DHA were 0.9% and 4% of fatty acids, respectively. Erythrocyte EPA and DHA were significantly increased from baseline in the Omacor group (both $p < 0.001$), but did not change in the placebo group; at the end of the treatment period both were higher in the Omacor group (both $p < 0.001$). Mean liver fat at baseline was approx. 22%. There was a mean 30% decrease in liver fat from baseline with Omacor compared with a mean 9% decrease in the placebo group. In multivariable linear regression modelling (intention to treat (ITT) analyses) adjusting for baseline measurement and other confounders there was a strong trend towards a decrease in liver fat with Omacor compared to placebo (Beta -3.6 (95% CI -8.0, 0.8), p=0.1). In secondary ITT analyses, after adjustment for the same factors in the model, % enrichment with DHA was independently associated with a decrease in liver fat (Beta -1.7 (95% CI -2.9, -0.5), p=0.007). There were no improvements in the liver fibrosis biomarker scores or CK-18 with Omacor. Long chain omega-3 fatty acids decrease liver fat in patients with NAFLD and high DHA enrichment may be especially important for achieving this. Research supported by the National Institute for Health Research

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Impact of long chain n-3 PUFA and flavanols on non-alcoholic fatty liver disease 02.18.1430.004

Presenter Last Name: **Minihane**

Impact of long chain n-3 PUFA and flavanols on non-alcoholic fatty liver disease D Vauzour1*, I Rodriguez-Ramiro1*, S Rushbook2, D Bevan3, J Gavrilovic3, S Davis4, N Tejera-Hernandez1, AM Minihane1. 1Norwich Medical School, University of East Anglia (UEA), UK; 2Gastroenterology, Norfolk and Norwich University Hospital, UK; 3Biological Sciences, UEA; 4Pancreatic Cancer Centre, Cambridge, UK *Joint first author Non-alcoholic fatty liver disease (NAFLD), a co-morbidity of obesity and major cardiovascular disease risk factor, is now the most common liver disorder in western societies. The pathological progression is characterised by a two hit process of hepatic lipid accumulation (1st hit) and oxidative stress and inflammation (2nd hit). Here using the high-fat high-fructose (HF/HFr) mouse model of NAFLD, the individual and combined impact of 16 weeks intervention with the fish oil n-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)(25mg per day, FO) and cocoa flavanols (2.5mg per day, FLAV) on NALFD was established. NAFLD severity of hepatic tissue was characterised using the human histology NAFLD activity score (NAS). A significant impact on hepatic steatosis (fat accumulation)($P<0.001$) and

hepatocyte ballooning was evident (P<0.001), with mean (SEM) values of 0 (0), 1.05 (0.25), 0.22 (0.14), 0.15 (0.05) and 0.10 (0.10) and 0 (0), 1.60 (0.22), 0.56 (0.23), 0.20 (0.06) and 0 (0) in the low fat, HF/HFr, HF/HrF+FLAV, HF/HFr+FO and HF/HFr+FLAV+FO animals. The greatest benefit was evident following the FO+FLAV intervention. Significant impacts of treatment on body and liver weights, insulin sensitivity as determined by an intra-peritoneal glucose tolerance test, and on gene expression and protein levels of key determinants of hepatic lipid metabolism and inflammation were also observed (P<0.01), which provide significant insight into the improved clinical scores. Thus a combined FO+FLAV emerged as a highly effective strategy to prevent NAFLD in a mouse model, which now warrants further establishment in human NAFLD patients.

N-3 Polyunsaturated fatty acids reduce metabolic endotoxemia and prevent metabolic disorders associated to obesity: a fat-1 transgenic mouse and Caco-2/TC7 cell study. 02.18.1430.005

Presenter Last Name: **Bidu**

BACKGROUND- Western-type diet (high-fat/high-carbohydrates) impacts on intestinal microbiota, leading to 'leaky gut', increase intestinal permeability, bacterial translocation, and elevated plasma lipopolysaccharide (LPS) levels (metabolic endotoxemia). N-3 fatty acids (n-3 FA) are known to prevent obesity development, but mechanisms are not fully understood. OBJECTIVE- We aimed to determine whether n-3 FA protect from dietary obesity by alleviating metabolic endotoxemia, possibly through the preservation of the integrity of the gut barrier. For that, we used the fat-1 transgenic mouse model and Caco-2/TC7 enterocyte-like cells. METHODS- Fat-1 transgenic mice encode a n-3 FA desaturase from C. elegans and are able to endogenously convert n-6 to n-3 PUFAs. Fat-1 mice and wild-type littermates were fed a high-fat/high-sucrose (HF/HS) or a control diet during 18 weeks. Plasma and jejunum were collected for biochemical and histological analyses. In parallel, Caco-2/TC7 cells were cultured under various FA and endotoxin conditions for protein, transcript and physiological analyses. RESULTS- When fed a HF/HS diet, fat-1 mice did not develop obesity and showed significantly lower plasma LPS levels as compared to wild-type. Moreover, jejunal integrity and permeability were better preserved in fat-1 mice than in wild-type. Finally, at both transcript and protein levels, expression of tight junction proteins was lower in wild-type mice compared to fat-1. Caco-2/TC7 studies revealed higher cellular integrity when treated with n-3 FA. Expression of tight junction proteins was decreased by a LPS, but increased by n-3 FA treatment. A pretreatment of Caco-2/TC7 with n-3 FA alleviates LPS effects on tight junction. CONCLUSIONS- N-3 FA protect fat-1 mice from diet-induced obesity in part by alleviating intestine integrity and permeability disruption, resulting in a prevention of metabolic endotoxemia associated with obesity-related dysmetabolism. Concordant observations were made on cultured

Caco-2/TC7, with direct evidence for a role of n-3 FA in maintaining tight junction integrity.

Transfer of omega-3 fatty acids across the blood–brain barrier after dietary supplementation with a docosahexaenoic acid (DHA)-rich omega-3 fatty acid preparation in patients with Alzheimer's disease: the OmegAD study 02.19.1430.001

Presenter Last Name: **Palmblad**

Objective. Little is known about the transfer of essential fatty acids (FAs) across the human blood–brain barrier (BBB) in adulthood. In this study we investigated whether oral supplementation with omega-3 (n-3) FAs would change the FA profile of the cerebrospinal fluid (CSF). Methods: A total of 33 patients (18 receiving the n-3 FA supplement and 15 receiving placebo) were included in the study. These patients were participants in the double-blind, placebo-controlled randomised OmegAD study in which 204 patients with mild Alzheimer's disease (AD) received 2.3 g n-3 FA [high in docosahexaenoic acid (DHA)] or placebo daily for 6 months. CSF FA levels were related to changes in plasma FA and to CSF biomarkers of AD and inflammation (total and phosphorylated tau, sIL-1R2 etc). Results: At 6 months, the n-3 FA supplement group displayed significant increases in CSF (and plasma) eicosapentaenoic acid (EPA), DHA and total n-3 FA levels ($P < 0.01$) whereas no changes were observed in the placebo group. Changes in CSF and plasma levels of EPA and docosapentaenoic acid (22:5 n-3) were strongly correlated (in contrast to those of DHA). Thus, the more the FA increased in plasma, the more they were enhanced in CSF. Changes in DHA levels in CSF were inversely correlated with CSF levels of total and phosphorylated tau, and directly correlated with soluble interleukin-1 receptor type II. Thus, the more DHA increased in CSF, the greater the change in CSF AD/inflammatory biomarkers. Conclusions: Oral supplementation with n-3 FAs conferred changes in the n-3 FA profile in CSF, suggesting transfer of these FAs across the BBB in adults. ClinicalTrials.gov Identifier: NCT00211159.

The role of endocannabinoid signalling in the divergent effects of eicosapentaenoic acid and docosahexaenoic acid in neural stem cell fate 02.19.1430.002

Presenter Last Name: **Dyall**

The endocannabinoid system acting via CB1 and CB2 receptors plays a key role in regulating neural development and adult neurogenesis, and potentially brain repair. The omega-3 fatty acids eicosapentaenoic acid (EPA) and

docosahexaenoic acid (DHA) also play a crucial role in neuroprotection and stimulate adult neurogenesis, and may possess therapeutic potential in a variety of neurodegenerative conditions. There is emerging evidence of a complex interplay between the endocannabinoid system, omega-3 fatty acids and the immune system in the promotion of brain self-repair. This study investigated the effects of EPA or DHA on neural stem cell (NSC) fate and the role of CB1 and CB2 receptor signalling pathways in these effects. Experiments were performed in NSCs from wild type (C57BL6/J, WT) and interleukin-1b knock-out (IL-1b KO) mice. In NSCs from WT mice, EPA, but not DHA significantly increased cell proliferation compared to controls (10 nM, $P<0.001$). Blocking CB1 (AM251, 1 μM) and/or CB2 (AM630, 1 μM) abrogated the effect of EPA, suggesting the effect was mediated via CB1 and CB2 receptor signalling pathways. Further investigation into the signal-regulated mitogen-activated protein kinases (MAPK/ERK1 and MAPK/ERK2, p44, p42), stress-activated protein kinases c-jun N-terminal kinase (SAPK/JNK) and p38 kinase signalling cascade revealed significant differences between the effects of EPA and DHA. In NSCs from IL-1b KO mice the effects of EPA were reversed and proliferation was significantly reduced compared to controls ($P<0.001$), whereas DHA significantly increased proliferation ($P<0.001$), suggesting the effects are mediated by endogenous IL-1β Furthermore, in these cells EPA and DHA also showed significant differences in the activation of the MAPK cascade. These results provide crucial new insights into the divergent effects of EPA and DHA in NSC proliferation and the pathways involved, and also highlight the therapeutic potential of their interplay with endocannabinoid signalling in neurogenesis and potentially brain repair.

Fatty acids and sleep in UK children: Subjective and pilot objective sleep results from the DOLAB study – A randomized controlled trial 02.19.1430.003

Presenter Last Name: **Richardson**

Sleep problems in children are associated with poor general health, behavioural and cognitive problems, as are deficiencies of omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) such as docosahexaenoic acid (DHA). Theory and some evidence support a role for LC-PUFA in sleep regulation, but this issue has received little formal investigation. We examined associations between blood fatty acid concentrations (from fingerstick blood samples) and subjective sleep (using an age-standardised parent questionnaire) in a large epidemiological sample of healthy children aged 7-9 years (n=395) from mainstream UK schools. In a randomised controlled trial, we then explored whether 16 weeks of dietary supplementation with algal-source DHA (600mg/day) vs. placebo might improve sleep in a subset of those children (n=362) who were underperforming in reading. In a randomly selected subsample (n=43), sleep was also assessed objectively via actigraphy. In 40%

of the epidemiological sample, Child Sleep Habits Questionnaire scores indicated clinical-level sleep problems. Furthermore, poorer total sleep disturbance scores were weakly but significantly associated with lower blood DHA concentrations (std. coeff. -0.105*) and a lower ratio of DHA to arachidonic acid (AA) (std. coeff. -0.119**). The treatment trial showed no significant effects on subjective sleep measures. However in the small actigraphy subsample, dietary supplementation with DHA led on average to 7 fewer wake episodes and 58 minutes more sleep per night. Cautiously, we conclude that higher blood concentrations of DHA may relate to better child sleep, as rated by parents. Exploratory pilot objective evidence from actigraphy suggests that dietary supplementation with DHA may improve children's sleep, but further investigations are needed to confirm this.

AT-RvD1 modulates synaptic plasticity and prevents neuroinflammation in a mouse model of surgery-induced cognitive decline. 02.19.1430.004

Presenter Last Name: **Terrando**

Impairment of cognition is a frequent complication of surgery and acute illness. Surgical trauma launches a cascade of inflammatory events that can lead to neuroinflammation and cognitive decline. Herein we report for the first time how treatment with aspirin-triggered resolvin D1 (AT-RvD1) counter regulates deleterious effects of exacerbated inflammation within the brain, which contributes to cognitive decline. 12-wk-old male C57BL/6 were randomly assigned as follows: 1) untreated control animals with analgesia, 2) surgery (an open tibial fracture of the left hind leg with intramedullary fixation) under isoflurane general anesthesia and postoperative analgesia, 3) surgery with preemptive AT-RvD1 treatment (IP bolus, 100 ng dose per mouse) or 24h delayed, or 4) AT-RvD1 alone. Separate cohorts of animals were used to perform electrophysiology, systemic and central inflammatory changes including astrocytes (GFAP) immunofluorescence, and hippocampal-dependent cognition using trace fear conditioning (TFC). Systemic inflammatory markers were significantly reduced by prophylactic administration of AT-RvD1 as early as 6h ($P<0.001$). 24h after surgery in the CNS we found distinct changes in astrocytes activation and morphology marked by enlarged cell bodies and reduced filaments. Remarkably, systemic administration of AT-RvD1 restored astrocytic dysfunction. Reactive astrogliosis was further associated with impaired pre-synaptic mechanism (PPF) and long-term potentiation (LTP) deficit starting at 24h and peaking 72h postoperatively. Treatment with AT-RvD1, both pre- or 24h postoperatively, improved neuronal function. At 72h, surgical animals were also tested with TFC to assess hippocampal-dependent memory function and further displayed memory dysfunction that was fully reverted by AT-RvD1 ($P<0.001$). Overall, peripheral surgery affects synaptic transmission and plasticity causing postoperative cognitive decline. Administration of specialized proresolving

mediators, including AT-RvD1, effectively modulates this inflammatory sequelae and restore neuronal-glial function after trauma.

The role of polyunsaturated fatty acids mediators in the resolution of neuroinflammation 02.19.1430.005

Presenter Last Name: **Trépanier**

Introduction: Resolution of inflammation is controlled by polyunsaturated fatty acid (PUFA) bioactive mediators. To date, however, the time-course and role of PUFA in resolution of neuroinflammation has not been characterized. Objective: The objective of this study is to characterize neuroinflammation in a self-resolving intracerebroventricular (i.c.v.) lipopolysaccharide (LPS) model and to determine the effect of modifying brain docosahexaenoic acid (DHA)on the time-course of resolution of neuroinflammation and the production of pro-resolving mediators. Methods: c57bl/6 mice received LPS in the left lateral ventricle. Animals were euthanized at various time points (ranging from 12 hours to 28 days) for immunohistochemistry, microarray, and lipidomic analysis. A fourth set of injected animals was tested in the y-maze at 7 days post-surgery. In order to test the effect of modifying brain DHA, the Fat-1 transgenic mouse (endogenously produces n-3 PUFA from n-6 PUFA) was compared to its wildtype littermate on either a 2% fish oil or -3 PUFA deficient diet in the i.c.v. LPS model. Results: Peak microglia activation was observed at 7 days post-surgery and returned to baseline at 21 days following surgery. Based on this cellular event, the resolution index (time from maximal microglia activation to 50%) was determined to be 6 days. Samples for gene expression and bioactive lipid mediator data have been collected. Data will be presented at ISSFAL. At 7 days post LPS injection (maximal inflammation), pilot data (n=11) suggest a trend ($p = 0.06$) that LPS treated animals showed a decreased performance in the y-compared to non-treated animals, suggesting a cognitive deficit. The fish oil supplementation and Fat-1 study is ongoing. Data will be presented at ISSFAL. Conclusion: This work will define neuroinflammation and its resolution as well as identify a potential role for DHA in modulating neuroinflammation through the production DHA-derived pro-resolving mediators.

Bile acids and lipid metabolism 03.20.0945.001

Presenter Last Name: **Angelin**

Awaiting final submittal

Serum triglyceride to HDL ratio and its relationship to insulin resistance among 5-15 year old Sri Lankan children.

03.20.0945.002

Presenter Last Name: **Wickramasinghe**

Introduction Prevalence of Insulin resistance (IR) among south Asian populations is high and is increasing with the obesity epidemic experiencing in this region. Determining insulin resistance is costly. Triglyceride/high-density lipoprotein (TG/HDL) ratio has shown to be a good proxy marker for IR in adults, but is not yet well defined in children. Objective: To identify TG/HDL ratio as an IR marker in 5-15year old school children in Colombo district of Sri Lanka. Materials and Methods: After a 12hour overnight fast, blood was drawn for lipid profile, fasting blood sugar and insulin. Height, weight, waist circumference (WC), fat mass(FM) and blood pressure (SBP & DBP) was measured. Insulin resistance was evaluated using HOMA-IR. Results Data from 309 children were analysed (boys 133). 13(4.2%) were obese and 35(11.3%) were overweight according to IOTF classification. The mean(sd) HOMA-IR was 1.1(1.1) and 0.94(1.2) for girls and boys respectively. The 4th quartile value of HOMA-IR for the whole population was 1.2 and in obese children 2.26. Mean(sd) TG/HDL ratio of whole population was 1.87(1.1) and in obese children 3.0(1.6). TG/HDL ratio was significantly correlated with HOMA-IR, BMI SDS, waist-to height ratio, SBP, DBP and fat mass index. 36 children with IR (HOMA-IR >1 SD above the mean, i.e. >2.16) had significantly higher TG/HDL (2.68 ± 1.64) compared to non-IR (n=273) children (1.76 ± 0.93). Children with TG/HDL ratio >3 were significantly heavier and had high SBP and DBP, HOMA-IR, and lower HDL levels. Although FBS, cholesterol and LDL were higher in TG/HDL ratio >3 group, they were statistically not significant. Conclusions TG/HDL ratio is correlated with IR among school children in Colombo district. Further studies are required to determine TG/HDL ratio cut-off value as proxy of IR among South Asian school children.

Association of Erythrocyte Long-Chain ω-3 Fatty Acids and Long-term Clinical Outcome – The Ludwigshafen Risk and Cardiovascular Health Study 03.20.0945.003

Presenter Last Name: **von Schacky**

Introduction Observational studies have shown inverse associations between EPA+DHA in the diet or measured as blood levels and cardiovascular risk, whereas randomized controlled trials with EPA+DHA supplementation had mixed results. Therefore we aimed to investigate the association of EPA+DHA levels in erythrocytes with mortality in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study, a cohort with medium to high cardiovascular risk. Methods

The fatty acid composition of erythrocytes in 3259 patients of the LURIC study was analyzed using the HS-Omega-3 Index® methodology. Association of EPA+DHA with clinical outcomes was investigated with Cox proportional hazards regression. Results EPA and DHA ranged from 0.18-3.64% and 2.03-10.17% of total fatty acids in erythrocytes with a mean (SD) of 0.76% (0.31) and 5.07% (1.09), respectively. Higher concentration of EPA was associated with higher LDL-C and HDL-C as well as lower triglycerides (TG), fasting glucose (FG), hsCRP, NT-proBNP and TnT. In contrast, DHA was associated only with lower TG and FG, but with higher NT-proBNP. The highest tertile of EPA compared to the lowest tertile was associated with reduced all-cause mortality and cardiovascular mortality with HRs of 0.75 (0.64-0.88) and 0.70 (0.58-0.86) in models adjusted for conventional risk factors, respectively. In contrast, there was no association of DHA to all-cause mortality or cardiovascular mortality. Conclusion Higher EPA concentration in LURIC was associated with changes in concentrations of other lipid fractions, mainly lower TG, lower FG, lower markers of inflammation and of heart damage, and was associated with a lower risk for all-cause mortality as well as cardiovascular mortality. In contrast, DHA was positively associated with NT-BNP and showed no association with fatal endpoints.

Apolipoprotein E epsilon 4 genotype and Docosahexaenoic acid metabolism: data from mice and humans 03.20.0945.004

Presenter Last Name: **Plourde**

Background: Over the last five years, our group investigated imbalance in the metabolism of docosahexaenoic acid (DHA) in humans and in transgenic mice carrying human apolipoprotein E epsilon 4 (APOE4+) genotype. One of our hypotheses is that rebalancing DHA metabolism could contribute to lower the risk of cognitive decline in APOE4+. Objective: To overview evidences collected from human and mice studies on disturbed DHA metabolism in APOE4+ compared to APOE4-. Results: In 2009, data obtained from clinical trial showed that in APOE4+, DHA concentration in plasma triglycerides was higher than APOE4-, but after a n-3 fatty acid supplementation, increase of DHA was lower than APOE4-. Using 13C-DHA, APOE4+ had 31% lower 13C-DHA in postprandial compared to APOE4-. However, we recently performed additional analysis from the SATgene studies and reported no differences in DHA of APOE4+ compared to APOE4-, potentially because of the younger age of the participants compared to our first studies. Our recent results in 4 and 13 month-old transgenic mice carrying human APOE4+ showed that brain uptake of 14C-DHA was 24% lower in APOE4+ than APOE2 but cortex DHA was significantly lower in 13 month-old mice only. Plasma DHA was significantly higher in APOE4 mice than APOE2 suggesting that lower brain uptake was not because of lower availability of DHA in plasma. Recently, we fed a diet containing 0.5 g/100g DHA

to APOE4+ mice and found that cognitive deficits were absent compared to other genotypes but when feeding a control or a high fat diet, APOE4+ mice had spatial and visual cognitive deficits compared to other genotypes. Conclusion: Disturbed DHA metabolism in APOE4+ seems highly age-dependant. However, a diet rich in DHA seems to prevent cognitive deficits in APOE4+ mice and is therefore a potential promising way for prevention of cognitive decline in this population.

Increases in whole body cholesterol synthesis and plasma clearance rates in sitosterolemia patients treated with ezetimibe

03.20.0945.005

Presenter Last Name: **Othman**

Sitosterolemia (STSL) is an inherited sterol storage disease manifested by very high plasma plant sterol (PS) levels with normal to moderately elevated plasma total cholesterol (TC). Whole body cholesterol synthesis is reduced in STSL, which may increase cholesterol absorption, and further contribute to whole body sterol retention. We studied the effects of ezetimibe (EZ), a sterol-absorption inhibitor, on plasma sterol levels and cholesterol absorption and synthesis in STSL. STSL patients (pts, n=8) were taken off EZ for 14 wks, wherein at wk 4 they received 1 mg/kg BW of intravenous O18-cholesterol and oral 13C-cholesterol, to measure cholesterol absorption, and oral (0.5 g/kg BW) deuterium-oxide to measure cholesterol fractional and absolute synthesis rates (FSR and ASR). Afterward, pts resumed EZ and the experiment repeated. Cholesterol and deuterium enrichments were measured by LC-MS and GC-IRMS, respectively, and sterols by GC. Data (mean±SEM) were analyzed by linear mixed-model. EZ substantially decreased plasma PS (sitosterol:-35±4% and campesterol:-47±4%, $p<0.0001$) levels, but only moderate reduction of TC (–12±6%, $p=0.09$). Serum LDL-C decreased (-23±6%) and HDL-C increased (26±8%, $p<0.05$) with EZ compared with off. EZ reduced cholesterol absorption (-47±7%) and increased FSR and ASR (>2-fold, all $p<0.01$). Cholesterol production rate (PR), reflecting absorbed and synthesized cholesterol, increased (29±16%, $p=0.15$) on EZ, correlating with increases in FSR and ASR (all $r=0.94$, $p<0001$). Metabolic clearance rate of cholesterol from plasma increased from 222±33 to 447±109 ml/d, correlating with increases in FSR ($r=0.93$), ASR ($r=0.91$) and PR ($r=0.94$, all $p<0.01$), and decrease in plasma TC ($r=-0.73$, $p=0.04$), suggesting increased whole body cholesterol turnover on EZ. Overall, EZ reduced plasma sterol levels and cholesterol absorption, while increasing cholesterol synthesis. EZ may work to improve cholesterol metabolism in STSL pts by enhancing cholesterol clearance and total body synthesis of cholesterol, which may have implications in the development of xanthomas and premature atherosclerosis.

Ethyl ester Versus Triglyceride formulations of long-chained omega-3 fatty acids: effect on non-fasting plasma triglycerides in moderate hypertriglyceridemia – a randomized controlled clinical trial 03.20.0945.006

Presenter Last Name: **Hedengran**

Aim: To compare the effects of n-3 poly-unsaturated fatty acids (n-3 PUFA) provided as triglyceride or as ethyl ester formulations on non-fasting plasma triglyceride levels. In contrast to fasting triglyceride in plasma, elevated non-fasting is directly associated with cardiovascular risk in both men and women. Methods: Patients (n=119) with moderate non-fasting hypertriglyceridemia (1.7-5.65 mmol/L) were recruited and randomized to either n-3 PUFA as a triglyceride or as an ethyl ester formulation or to an olive oil placebo at 4 g/day. Changes in non-fasting triglyceride level after 4 and 8 weeks of treatment were compared. Results: The median age was 64 years, 79 % were males, and 75 % used statins. Non-fasting plasma triglyceride levels as median (interquartile range) in mmol/L decreased in the triglyceride formulation group from 2.79 (1.12) to 1.81 (0.82) ($p<0.001$) and in the ethyl ester group from 2.70 (1.39) to 2.10 (1.23) ($p<0.001$). There was no change in the placebo group from 2.46 (1.38) to 2.69 (1.62) $p=0.14$). The decrease in plasma triglyceride was not significantly different between the two n-3 PUFA groups ($p=0.52$). The triglyceride lowering effect was present after 4 weeks of treatment. Conclusion: Non-fasting plasma triglyceride – a significant cardiovascular risk factor - decreased in median 20 – 35 % after supplementation with 4 grams/day for 8 weeks of triglyceride or ethyl ester based n-3 PUFA formulations. Anne Hedengran1, Jørn Dyerberg1, Pal Bela Szecsi1,William S. Harris2, Steen Stender1, 1 Department of Clinical Biochemistry, Copenhagen University Hospital, Gentofte, Denmark 2 Health Diagnostic Laboratory, Inc. Richmond, VA, USA

Roles of Some Endogenous Lipid Mediators in Cellular Defence against Oxidative Stress-Induced Carcinogenesis 03.21.0945.001

Presenter Last Name: **Surh**

One of hallmarks of chronic inflammation is the presence of infiltration of inflammatory cells and sustained production of pro-inflammatory mediators, which coordinately constitute cancer-prone microenvironment. Inflammatory cell-derived reactive oxygen/nitrogen species (ROS/RNS) can cause DNA damage/mutation, if accumulated in excess, thereby initiating carcinogenesis. Chronic inflammation also induces tissue remodeling including metaplasia,

angiogenesis, fibrosis and granulation which are implicated in tumor promotion and progression. In fibrotic tissues, fibroblast proliferation is stimulated, and components of extracellular matrix and pro-inflammatory factors are excessively produced and deposited, thereby accelerating tumorigenesis. A key enzyme that catalyzes the production of a distinct set of prostaglandins in response to inflammatory stimuli is cyclooxygenase-2 (COX-2). However, COX-2 contributes not only to the pro-inflammatory process, but also to the production of anti-inflammatory substances. For instance, 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) is one of the principal COX-2-derived prostaglandins has been known to display multifaceted cellular functions, including anti-oxidative, anti-inflammatory and other antitumorigenic effects. Many of the biological effects induced by this cyclopentenone prostaglandin involve direct modulation of redox-transcription factors and their regulators as the potential targets. Resolution of inflammation is an active process regulated by several endogenous anti-inflammatory and pro-resolving mediators (e.g., resolvins, protectins, lipoxins, etc.), rather than passive termination of the inflammatory response. Docosahexaenoic acid (DHA) is a precursor of D-series resolvins. Our recent studies have demonstrated that DHA enhances the efferocytic activity of macrophage, an essential event in resolution of inflammation. Topical application of DHA also inhibited UVB-induced oxidative and inflammatory damage and carcinogenesis in mouse skin.

Docosahexaenoic acid (DHA) mixed with extra virgin olive oil significantly reduces liver oxidative stress in high fat- induced liver steatosis in mice 03.21.0945.002

Presenter Last Name: **Rodrigo**

INTRODUCTION: Insulin resistance (IR), liver oxidative stress (LOE) and inflammation (IM) are factors which trigger non-alcoholic fatty liver disease (NAFLD) which is also characterized by a drastic reduction of docosahexaenoic acid (DHA). DHA is a ligand of the transcription factor PPAR-α, controlling liver lipid metabolism by stimulating lipolysis and inhibiting lipogenesis. Extra virgin olive oil (EVOO) has antioxidant, anti-inflammatory and cardio-protective actions. OBJECTIVES: Evaluate the protective effect of DHA mixed with EVOO against liver steatosis induced by a high fat diet (HFD) in mice. METHODOLOGY: Male mice C57BL/6J (n=9 each group) were fed 12 weeks with: i) control diet (CD), (proteins 20%, carbohydrates 70% and lipids 10%); ii) CD + DHA (from microalgae, 75 mg/kg/day); iii) CD + EVOO (100mg/kg/day); iv) CD + (DHA + EVOO); v) HFD (proteins 20%, carbohydrates 20%, lipids 60%) ; vi) HFD + DHA; vii) HFD + EVOO; and viii) HFD + (DHA + EVOO), were assessed for i) IR (HOMA); ii) LOE (total glutathione, GSH, GSSG, protein carbonylation, F8-isoprostanes); iii) IM (TNF-α, IL-1β, IL-6); iv) fatty acid profile of liver phospholipids (gas-chromatography); v) liver steatosis (histology, triglycerides and free fatty acids content); vi) mRNA for PPAR-α,

ACOX1 , CAT-1 and vii) PPAR-α binding activity to DNA. RESULTS: Group v) showed a significant increase ($p<0.05$, unifactorial ANNOVA and Newman Keuls tets) of IR, LOE, IM and liver steatosis, together with a significant reduction of DHA, mRNA (PPAR-α, ACOX1 and CAT-1), and the binding activity PPAR-α to DNA, compared to group i); group viii) showed no differences to controls. CONCLUSIONS: Administration of DHA + EVOO to mice prevent IR, LOE, IM and liver steatosis, being the activation of PPAR-α and the normal levels of mRNA for ACOX1 and CAT-I the possible mechanism for the protective effects.

Leptin induces in vitro and in vivo a lipid peroxidation and an inflammatory response, in neoplasicmammary epithelial cells

03.21.0945.003

Presenter Last Name: **Mahbouli**

Background: Obesity, frequently associated with hyperleptinemia, promotes the recurrence of breast cancer and increases mortality risk. Furthermore, oxidative stress and inflammation characterised by infiltration of immune cells into adipose tissue are described. This is associated with a lipid peroxidation and the production of bio-active compounds including isoprostanes. Objectives: This study aimed to evaluate in vitro and in vivo the impact of leptin on inflammatory response. In vitro evaluation was performed on HMEC, MCF-7 and MDA-MB-231 cells in presence of leptin (10 /100 ng/ml) by quantification of hydroperoxides (LOOH) and isoprostanes in culture media (spectrophotometry and RIA); by gene expression and catalytic activity of glutathione peroxidase 1 (Gpx1) and cyclooxygenases (COX2). In vivo inflammatory response of C57/BL6 mice syngenic mammary cancer model (EO771) was characterised: from plasma by quantification of interleukins (MILLIPLEX) and isoprostanes (RIA); from tumors by immunohistochemistry and western blotting (COX2, leptin) and by immunostaining of isoprostanes. Results: In vitro, whatever the leptin concentration, a slight increase of total cellular ROS production was observed. In HMEC, an induction of Gpx1 expression at 1h and catalytic activity at 6h, without increase of lipid peroxidation (LOOH: 679 ± 248 µmol/l), was observed. Inversely, in cancer cells,Gpx1 was not activated what induced a sharp increase in lipid peroxidation (1014 ± 207 µmol/l LOOH) and overexpression of COX2 with accumulation of isoprostanes($p<0.05$). These results were in agreement with in vivo model for which a relative increase of leptinemia (0.94 ± 0.51 vs 1.07 ± 0.46 ng/ml, $p<0.05$) was associated to an enhancement of inflammatory cytokines (TNFα, Il-6). Furthermore in mice tumors,COX2, leptin and isoprostanes amounts were increased together($p<0.05$). Conclusions:This study confirms the impact of leptin on lipid peroxidation and inflammatory response in cancer cells associated to the aggressivity state. These results contribute to support the evidence based link between obesity and breast carcinogenesis.

Lipid peroxidation and its relevance to pheromone production in marine fish under oxidative stress 03.21.0945.004

Presenter Last Name: **Lee**

Pheromones are lipid metabolites from polyunsaturated fatty acids secreted outside by an individual and received by a second individual. It serves for communication within species and may affect the behavior and developmental process of a species. Acute oxidative stress by hydrogen peroxide exposure was found to alter lipid metabolism in medaka fish by our research group. However, the effect of oxidative stress induced by hyperoxia and hypoxia on lipid metabolism is not well known. Adult male and female marine medaka fish (Oryzias melastigma) were exposed to hypoxia, normoxia and hyperoxia condition for 1 hour. Concentration of pheromones (Prostaglandin-F2α, 15-keto-Prostaglandin-F2α, 13,14-dihydro-15-keto-prostaglandin-F2α and 17α,20β-Dihydroxy-4-pregnen-3-one) and oxidized lipid products of arachidonic, adrenic, docosahexaenoic and eicosapentaenoic acids were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) in the fish muscles. Concentration of the Prostaglandin-F2α and 17α,20β-Dihydroxy-4-pregnen-3-one were significantly higher in male than female medaka muscle. Hypoxia condition reduced Prostaglandin-F2α and15-keto-Prostaglandin-F2α concentrations compared to normoxia whereas hyperoxia condition reduced 13,14-dihydro-15-keto-prostaglandin-F2α concentration in both male and female medaka. Concentration of oxidized lipid products showed gender difference and was lower in female (F2-isoprostanes, F2-dihomo-isoprostanes, dihomo-isofurans and F4-neuroprostanes) than male medaka. Compared to normoxia, hyperoxia environment increased F2-isoprostanes in both male and female medaka muscle. However hyperoxia environment elevated F2-dihomo-isoprostanes and dihomo-isofurans in female medaka only and suppressed in male medaka compared to normoxia. F3-isoprostane, neurofurans, RvE1 and RvD1 levels were low in both hypoxia and hyperoxia environment compared to normoxia and were also more apparent in female medaka muscle. Pheromones and F2-isoprostanes share the same precursor fatty acid (arachidonic acid). Our study indicates changes in oxygen tension suppress pheromone release and potentiate non-enzymatic lipid peroxidation of arachidonic acid as well as adrenic acid (an elongated product of arachidonic acid). Such outcome could affect reproductive system of marine fish and disrupt lipid metabolism.

Lipid Profiling Following Intake of the Omega 3 Fatty Acid DHA Identifies the Peroxidized Metabolites F4-Neuroprostanes as the Best Predictors of Atherosclerosis Prevention 03.21.0945.005

Presenter Last Name: **Gladine**

The anti-atherogenic effects of omega 3 fatty acids, namely eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) are well recognized but the impact of dietary intake on bioactive lipid mediator profiles remains unclear. Such a profiling effort may offer novel targets for future studies into the mechanism of action of omega 3 fatty acids. The present study aimed to determine the impact of DHA supplementation on the profiles of polyunsaturated fatty acids (PUFA) oxygenated metabolites and to investigate their contribution to atherosclerosis prevention. A special emphasis was given to the non-enzymatic metabolites knowing the high susceptibility of DHA to free radical-mediated peroxidation and the increased oxidative stress associated with plaque formation. Atherosclerosis prone mice (LDLR-/-) received increasing doses of DHA (0, 0.1, 1 or 2% of energy) during 20 weeks leading to a dose-dependent reduction of atherosclerosis ($R2=0.97$, $p=0.02$), triglyceridemia ($R2=0.97$, $p=0.01$) and cholesterolemia ($R2=0.96$, $p<0.01$). Targeted lipidomic analyses revealed that both the profiles of EPA and DHA and their corresponding oxygenated metabolites were substantially modulated in plasma and liver. Notably, the hepatic level of F4-neuroprostanes, a specific class of DHA peroxidized metabolites, was strongly correlated with the hepatic DHA level. Moreover, unbiased statistical analysis including correlation analyses, hierarchical cluster and projection to latent structure discriminate analysis revealed that the hepatic level of F4-neuroprostanes was the variable the most negatively correlated with the plaque extent ($p<0.001$) and along with plasma EPA-derived diols was an important mathematical positive predictor of atherosclerosis prevention. Thus, oxygenated n-3 PUFAs, and F4-neuroprostanes in particular, are potential biomarkers of DHA-associated atherosclerosis prevention. While these may contribute to the anti-atherogenic effects of DHA, further in vitro investigations are needed to confirm such a contention and to decipher the molecular mechanisms of action

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Fatty acid and oxylipin predictors of platelet function in adults with diabetes mellitus 03.21.0945.006

Presenter Last Name: **Block**

BACKGROUND: Enhanced platelet function places those with diabetes mellitus at very high risk for cardiovascular events. While research has found aspirin to be an effective antiplatelet aggregate, this relationship is not observed in many patients with diabetics. Since platelet function directly influences CVD risk, it is imperative that researchers determine what treatment options may be effective in individuals with diabetes. Fish oil has been investigated for its impact on platelet function but the mechanisms are unclear. METHODS: Using a sequential design with 10 subjects with type 2 diabetes mellitus, we investigated the effects

of aspirin and fish oil on platelet function and fatty acid metabolism. Plasma fatty acids and oxylipins were measured at baseline (prior to any treatment), after ingesting fish oil alone for 28 days, and then after 7 days of 81 mg/d of aspirin plus fish oil. Measures of platelet function (adenosine diphosphate [ADP]-whole blood induced platelet aggregation and thromboxane-B2) were measured after each treatment. Simple linear regression models were used and evaluated for best fit using Akaike information criterion (AIC). RESULTS: AIC criteria revealed changes in 22:5n6 (osbonic acid) and 20:0 (arachidic acid) as best predictors of changes in ADP and thromboxane-B2 dependent aggregation. PGF2a was also predictive of changes in thromboxane-B2 response. The change in mean levels of 22:5n6 showed a linear decrease from baseline to after fish oil only, and from fish oil only to fish oil plus aspirin. 20:0 and PGF2a indicated no linear trend; however, lower mean levels were present after the ingestion of fish oil plus aspirin. CONCLUSIONS: These results suggest that the modulating effects of fish oil plus aspirin on platelet function in adults with diabetes may be mediated by specific lipids. We propose further investigations of how altering the lipid milieu plays a major role in platelet function.

Effect of a Mediterranean diet intervention on 3T MRI-monitored carotid plaque progression and vulnerability. A sub-study of the PREDIMED trial. 03.22.0945.001

Presenter Last Name: **Sala-Vila**

Aim: The North-to-South gradient in coronary heart disease mortality could be explained in part by local dietary habits, such as adherence to the Mediterranean diet (MedDiet). We hypothesized that in older individual at high cardiovascular risk with carotid plaque, a MedDiet supplemented with either extra-virgin olive oil (MedDiet+EVOO) or mixed nuts (MedDiet+nuts) would induce changes on vessel wall volume and plaque composition (assessed by 3T magnetic resonance image [MRI]), toward less vulnerability compared to a control (low-fat) diet. Methods: Carotid ultrasound was performed in 245 participants in the PREDIMED trial of primary cardiovascular prevention with MedDiets (50% women, mean age 69 y). Subjects with plaques ≥2 mm thick (n=92, 38%) were invited to undergo MRI at baseline and after intervention for a mean of 1.8 years. Black-blood T1-weighted, T2-weighted, proton density-weighted images with fat and flow suppression and post gadolinium sagittal 3D MPRAGE and 2DT1 weighted images were obtained to evaluate vessel wall and plaque composition. Carotid bifurcation was covered by 9 slices with 2D sequences. Main outcomes were vessel wall volume, plaque components, total plaque volume and plaque index. Results: We evaluated 60 subjects with complete data (19 control diet; 20 MedDiet+EVOO; 21 MedDiet+nuts). In a multivariate model, after adjusting for sex, follow-up time and in-trial changes in statin treatment, changes in vessel wall volume (mm3) were (mean [95% CI]): 0 (-51 to 50) for the control group; -29 (-75 to 18) for the

MedDiet+Nuts group; and -98 [-145 to -51] for the MedDiet+EVOO group (p=0.024 versus control). No significant changes were observed in other outcomes. Conclusion: Compared to a control diet, consumption of a MedDiet supplemented with EVOO is associated with regression of carotid wall volume. The results contribute mechanistic evidence for the reduction of cardiovascular events observed in the arm supplemented with EVOO in the PREDIMED trial.

Dietary alpha-linolenic acid and long-chain n3PUFA: partners in prevention of all-cause mortality in individuals at high cardiovascular risk 03.22.0945.001

Presenter Last Name: **Sala-Vila**

Aim: Most evidence supporting the cardioprotective role of omega-3 fatty acids derives from studies of long-chain n-3 polyunsatutared fatty acids (LCn3PUFA), mainly found in fatty fish. Walnuts, soy and olive oil to a much lesser extent contain alpha-linolenic acid (ALA), the vegetable n3PUFA. Whether dietary ALA has cardioprotective effects on its own is a matter of dispute. We aimed to investigate the association between ALA intake and risk of both cardiovascular and all-cause mortality in individuals at high cardiovascular risk from Spain, a country with customarily high intakes of both ALA and LCn3PUFA. Design: We evaluated 4139 women and 3063 men aged 55-80 y enrolled in the PREDIMED trial. Nutrient consumption was ascertained by a validated food-frequency questionnaire and mortality by medical records and linkage to the National Death Index. Multivariable-adjusted Cox regression models (including meeting the AHA recommendation to consume ≥500 mg/d of LCn3PUFA for primary prevention) were fitted to estimate hazard ratios (HR) for mortality for each 1 g/d increase in ALA intake at baseline. Results: During follow-up >5 y, 431 deaths occurred (104 from cardiovascular disease; 32 from sudden cardiac death; 171 from cancer). For men, meeting the AHA recommendation (as occurred in 79.4% of the study population) was related to a borderline significantly reduced risk of total mortality (HR: 0.78; 95% CI: 0.58-1.05), fatal cardiovascular disease (HR: 0.56; 0.31-1.01) and sudden cardiac death (HR: 0.40; 0.16-1.04). For each additional daily gram of ALA, there was a 23% lower risk of total mortality (HR: 0.77; 0.64-0.93) and a lower 29% risk of fatal cancer (HR: 0.71; 0.52 to 0.97). No significant associations were found in women. Conclusion: Our study suggests that ALA contributes to reduction of total mortality and fatal cancer in men even when the background diet is high in marine-derived LCn3PUFA.

Role of milk fat globule membrane (MFGM) for modulating atherogenic plasma lipoproteins in humans: a randomized trial 03.22.0945.002

Presenter Last Name: **Rosqvist**

Background: Dairy fat is rich in saturated fat and generally raises plasma cholesterol levels that could increase the risk of coronary heart disease (CHD). However, different dairy products may have differential effects on lipoproteins, possibly due to the content of milk fat globule membrane (MFGM) enclosing the dairy fat. Objective: To investigate if the effect of dairy fat on plasma lipid levels is modulated by the presence of structurally intact MFGM. We hypothesized that MFGM-enclosed dairy fat would have a lower cholesterol-raising effect than dairy fat without MFGM. Design: This study was an 8-week single-blind, randomized, isocaloric trial with two parallel groups including overweight (mean BMI 28) adult men and women (n=46). All subjects consumed 40 g dairy fat per day either as cream (MFGM group) or butter (control group). Diets were matched for total fat, protein, carbohydrates and calcium. Subjects were not allowed to consume any other dairy products during the intervention. Results: The MFGM group and the control group showed differential responses in several lipoproteins with significantly higher levels of total cholesterol, LDL cholesterol, apolipoprotein B/A1 ratio and non-HDL cholesterol in the control group compared with MFGM group (all P-values<0.05). HDL cholesterol, total/HDL ratio, triglycerides, C-reactive protein, glucose and insulin were not significantly different between groups. Conclusions: Butter markedly increased atherogenic lipoproteins whereas cream did not, findings that may fully or partly be assigned to the presence of MFGM in these dairy foods. The present results could also explain some of the inconsistent associations between total dairy consumption and CHD risk found in cohort studies.

EPA and DHA in whole-blood are differentially and sex-specifically associated with cardio-metabolic risk markers in 8-11-year-old children 03.22.0945.003

Presenter Last Name: **Damsgaard**

Introduction n-3 long-chain polyunsaturated fatty acids improve cardiovascular risk markers in adults. These effects may differ between EPA (20:5n-3) and DHA (22:6n-3), but we lack evidence in children. Aim We aimed to 1) investigate associations between EPA and DHA in whole-blood and early cardio-metabolic risk markers in 713 children aged 8-11 years and 2) explore potential mediation through waist circumference and physical activity and dietary confounding by protein and fiber intake, using baseline data from the OPUS School Meal Study. Methods We collected data on parental education and pubertal stage, 7-day dietary records, physical activity by accelerometry and measured anthropometry, blood pressure, and heart rate. Blood samples were analyzed for whole-blood fatty acid composition, cholesterols, and triacylglycerol (TG), insulin resistance by

the homeostasis model of assessment (HOMA-IR), and inflammatory markers. Results Whole-blood EPA was negatively associated with TG (P=0.003) and positively with total cholesterol, LDL cholesterol, and HDL:TG (all P<0.01). EPA, and particularly DHA status, were associated with reduced heart rate (P=0.02 and P=0.002, respectively). Also, DHA was negatively associated with HOMA-IR (P=0.003) and tended to be negatively associated with a metabolic syndrome score (P=0.05). Diastolic blood pressure was not associated with EPA in girls [β=-1.6 mmHg (95% CI -4.4; 1.2)] (P=0.27), but increased 2.7 mmHg (95% CI 0.4; 5.1) per w/w% EPA increase in boys. Adjustment for waist circumference and physical activity did not change the associations, and the associations remained after adjustment for protein and fiber intake. Conclusion EPA status was beneficially associated with lipid profile whereas DHA was mainly associated with heart rate and insulin homeostasis in the children. The sex-specific associations with blood pressure confirm our previous findings and warrant further investigation. Funding The OPUS (Optimal Well-Being, Development, and Health for Danish Children through a Healthy New Nordic Diet) Centre was supported by the Nordea Foundation.

Docosahexaenoic fatty acid favorably alters inflammatory pathways and macrophage polarization in the aorta of atherosclerotic mice 03.22.0945.004

Presenter Last Name: **Gladine**

The omega 3 PUFA docosahexaenoic acid (DHA) has potent anti-atherosclerotic properties but its molecular action at the vascular level remains poorly explored. Knowing the broad range of DHA targets at the cellular and molecular levels, microarray analysis was chosen to evaluate the effect of DHA intake on the overall gene expression in the aorta of atherosclerosis prone LDLR-/- mice. Mice were fed (20 wks) an atherogenic diet and received daily oral gavages with either oleic acid-rich oil or a mixture of oils providing 2% of energy as DHA. As anticipated, DHA intake exerts a potent cardioprotective effects, i.e. reduced systolic blood pressure (-16 mmHg, p<0.01), plasma levels of cholesterol (-28%, p<0.001) and triacylglycerols (-37%, p<0.01), and lowered the extent of atherosclerosis measured in the aortic root (-35%, p<0.001). Bioinformatics analysis of microarray data (functional enrichment and canonical pathway analysis) of the aortic gene expression revealed that DHA supplementation mostly affected inflammatory processes and innate immunity, with a remarkable number of down-regulated genes associated with pro-inflammatory activity of immune cells (e.g. CCL5, CCR7), cell movement (e.g. ICAM-2, SELP, PECAM-1), and antigen presentation (e.g. HLA-DQA1, HLA-DRB1, HLA-DQB1, H2-Q8). Interestingly, the expression of several significantly modulated genes were identified as markers of macrophage phenotype, suggesting their preferential orientation towards a M2 reparative phenotype. This hypothesis was confirmed

by the immunohistological analysis of a specific biomarker of M2 macrophage (arginase 1), which abundance was increased in the aortic root of the DHA supplemented group (+111%, p=0.01). All together, these results suggest that DHA mainly intefere with atherosclerosis progression by limiting inflammation probably through the orientation of macrophages towards a M2 reparative phenotype.

Association of Trans Fatty Acids and Clinical Long-term Outcome – The Ludwigshafen Risk and Cardiovascular Health Study 03.22.0945.005

Presenter Last Name: **von Schacky**

Introduction Trans fatty acids (TFA) are produced by the food industry (IP-TFA) and also occur naturally in trace amounts in beef and dairy products. For the latter, beneficial health effects have been claimed, while there are numerous reports about IP-TFA being hazardous to human health. Therefore we aimed to investigate the association of TFA concentration measured in the membrane of erythrocytes with mortality in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Methods The fatty acid composition of erythrocyte membranes was analyzed using the HS-Omega-3 Index® methodology. Association of TFA with clinical outcome was investigated with Cox proportional hazards regression. Results TFA were measured in 3259 patients of the LURIC study ranging from 0.27-2.40% of total fatty acids in erythrocyte membranes with a mean (SD) of 0.96% (0.26). Higher concentration of TFA was associated with higher age, LDL-C and HDL-C as well as lower BMI, triglycerides, blood pressure and fasting glucose. Total TFA were associated with reduced mortality due to cardiovascular causes or sudden cardiac death. This risk reduction was mainly driven by an increase of C16:1n7t (trans-palmitoleic acid) which is a naturally occurring TFA from ruminants. The protective effect of high concentration of C16:1n7t for sudden cardiac death persisted after multivariate adjustment with HRs of 0.83 (0.61-1.11) and 0.63 (0.46-0.88) for the second and third tertile compared to the first tertile, respectively. Concentration of IP-TFAs were low, and not associated with increased risk for adverse outcomes. Conclusion Higher TFA concentration was associated with a healthier metabolic profile except for a slight increase in LDL-C. Higher TFA were protective with regard to cardiovascular mortality and sudden death. This was mainly driven by ruminant derived TFAs. The low IP-TFAs concentration we found in LURIC was comparable to current levels in the US, and posed no cardiovascular risk..

The Omega-3 Index in heart failure patients: associations with clinical data, comorbidities, and prognosis 03.22.0945.006

Presenter Last Name: **von Schacky**

Background The omega-3 index assesses the quantity of the fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the erythrocyte membrane. Higher levels of the omega-3 index have been shown to correlate with lower incidences of cardiovascular diseases and sudden cardiac death. Larger scale data on the distribution of the omega-3 index and its influencing factors are lacking in patients with heart failure. Methods In the Randomized Interdisciplinary Network for Heart Failure (INH), blood samples were obtained at baseline and stored at -80°C. Erythrocyte fatty acid composition was analyzed using a standardized and validated methodology. Results In 914 consecutive patients the omega-3 index could be determined. Mean age was 68±12 years, 72% were male, 43% were in NYHA class III or IV, and mean LVEF was 30±8%. The mean omega-3 index was 3.74±0.97%. The index showed weak associations with the following variables (all $p<0.03$): age, higher level of education, uncured malignancy, LDL:HDL cholesterol ratio, serum triglycerides, and haemoglobin. No association was found with ejection fraction, NYHA functional class, the underlying cause of heart failure, and markers of renal function or inflammation. Whether the omega-3 index confers significant prognostic will be evaluated until presentation of data. Interpretation In this large well-characterized cohort of patients with chronic systolic heart failure, the omega-3 index was markedly lower than in unselected German individuals and far from the range suggested to be optimal (8 – 11%). Furthermore, the omega-3 index was associated with an unconventional panel of established indicators of mortality risk in heart failure.

Lipid Membranes and insulin signaling 03.23.1430.001

Presenter Last Name: **Inokuchi**

Gangliosides expose sialic acid residue to outside of outer leaflet membranes, it is very interesting to know their true physiological counterparts for electrostatic interactions in membrane microdomains (lipid rafts). It has been proposed a working hypothesis ‘‘metabolic disorders, such as type 2 diabetes, are membrane microdomain disorders caused by aberrant expression of gangliosides”. Molecular pathogenesis of type 2 diabetes and insulin resistance focusing on the interaction between insulin receptor and GM3 ganglioside in adipocytes is discussed. It is expected that the development of novel diagnosis of metabolic syndrome by identifying the specific ganglioside species and a therapeutic strategy ‘‘membrane microdomain ortho-signaling therapy”.

Dose-dependent effects of thiazolidinediones in dietary obese

mice 03.23.1430.002

Presenter Last Name: **Svobodova**

Thiazolidinediones (TZD), the activators of PPARg, are used for treatment of insulin resistance in diabetic patients; however, the therapy is associated with unwanted side effects. The insulin-sensitizing action of TZD is thought to result from activation of PPARg in white adipose tissue, associated with the secretion of adiponectin and consequent stimulation of AMP-activated protein kinase (AMPK) in other tissues. Our previous results suggested that the effects of TZD, administered at sub-optimal doses with respect to the improvement of insulin sensitivity in mice, may involve a change in plasma lipid profile resulting from modulation of hepatic lipid metabolism, independent of the adiponectin–AMPK axis. The aim of the present study was to further characterize the dose-dependent mechanism of TZD action. B6 mice were randomly assigned to dietary treatment using: 1) a high fat diet (HF); 2) HF diet with “low” dose of rosiglitazone -10 mg/kg diet (HF+R10); 3) HF diet with “high” dose of rosiglitazone – 100 mg/kg diet (HF+R100). Rosiglitazone in a dose-dependent manner induced high molecular weight form of adiponectin in plasma. Only the HF+R100 mice exhibited significantly higher activity of hepatic AMPK a2 isoform. Conversely, the HF+R10 mice showed elevated hepatic lipid content, upregulated expression of genes associated with de novo lipogenesis, namely fatty acid synthase and D-9 desaturase 1(SCD-1). These mice also exhibited specific enrichment of palmitoleate in various liver lipid fractions and in total plasma lipids. Moreover, also expression of CIDEA, which is crucial for lipid droplet formation and inhibits AMPK, was the highest in the HF+R10 mice. In conclusion, we show that different mechanisms contribute to the insulin-sensitizing effect of TZD, depending on the dose of the drug. New strategies for treatment of diabetic patients may be based on the use of sub-optimal doses of TZD in combination with other interventions. Funding: Czech Science Foundation (P301/11/0226)

A DIET RICH IN OMEGA-6 POLYUNSATURATED FATTY ACIDS (OMEGA-6 PUFA) REDUCES SPONTANEOUS ACTIVITY IN MICE

03.23.1430.003

Presenter Last Name: **Ghosh**

Obesity, insulin resistance and the incidence of cardiometabolic disease continue to rise unabated in the western world. A lack of physical inactivity and a faulty diet are blamed as independent factors precipitating the rise in these disease states. However, a causative link between a lack of physical activity and recent changes in the 'western diet' has not been investigated . Over the last few decades, ω-6 polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid

(MUFA) content in the 'western diet' has increased manyfold at the expense of saturated fats. The goal of this study was to evaluate the impact of an ω-6 PUFA-rich and MUFA rich high fat (HF) diets on spontaneous voluntary activity and the development of insulin resistance in mice. 8-week old female C57/Bl6 mice were fed either a diet of ω-6 PUFA rich corn oil or MUFA rich olive oil for 6 weeks, with chow fed mice as a low-fat control. Although food and water intake, body weight gain and resting energy expenditure in the daytime stayed similar in mice fed HF diets, ω-6 PUFA-rich corn oil diet reduced spontaneous physical activity in mice during both the light and dark phases. Such depressed activity levels were also associated with a lower respiratory ratio in mice in the active (dark) phase. Corn oil fed mice also demonstrated hyperinsulinemia and impaired glucose disposal in response to intraperitoneal glucose tolerance and insulin tolerance tests, despite similar increases in fat mass after HF feeding compared to olive oil fed mice. If this data holds true for humans, this study presents a novel quantitatively important contribution of excess ω-6 PUFA intake to the loss of physical activity and the development of insulin resistance in the western world. Supported by Canadian Diabetes Association and UBC-O

Asian and Caucasian type 2 diabetes have different response to n-3 polyunsaturated fatty acids 03.23.1430.004

Presenter Last Name: **Li**

N-3 polyunsaturated fatty acids (PUFA), especially marine n-3 PUFA C20:5n-3 and C22:6n-3 intake, had been demonstrated to improve insulin sensitivity in animal models. However, observational studies in relation to the association of n-3 PUFA intake with risk of T2D were inconsistent. In addition, fish, rich in marine n-3 PUFA, also showed inconsistent associations with risk of T2D in observational studies. The aim of the present meta-analysis was to investigate the associations of fish and n-3 PUFA intake with risk of T2D based on prospective cohort studies. The differences of tissue (plasma/serum/erythrocytes) n-3 PUFA compositions in subjects with and without T2D were also investigated based on prospective cohort and case-control studies. Twenty-four studies including 24,509 T2D patients and 545,275 participants were identified. For cohort studies, the summary RR of T2D for the highest vs lowest categories of total fish, marine n-3 PUFA and alpha-linolenic acid intake was 1.07 (95% CI: 0.91, 1.25), 1.07 (95% CI: 0.95, 1.20) and 0.93 (95% CI: 0.81, 1.07), respectively. Subgroup analyses indicated that summary RR (highest vs lowest category) of T2D for fish and marine n-3 PUFA intake was 0.89 (95% CI: 0.81, 0.98) and 0.87 (95% CI: 0.79, 0.96) for Asian populations, and 1.20 (95% CI: 1.01, 1.44) and 1.16 (95% CI: 1.04, 1.28) for Western populations. Asian subjects with T2D had significantly lower tissue compositions of C22:6n-3 (SMD: 21.43; 95% CI: 21.75, 21.12) and total n-3 PUFA (SMD:

21.41; 95% CI: 22.23, 20.59) compared with those without T2D. This systematic review and meta-analysis provided evidence that marine n-3 PUFA consumption had protective associations with risk of T2D in Asian populations, but was positively associated with risk of T2D in Western populations. T2D subjects in Asian but not in Western countries had lower tissue C22:6n-3 and total n-3 PUFA compositions than healthy controls.

Lower Serum Non-Esterified Eicosapentaenoic Acid (EPA) is Associated with Insulin Resistance in the PROspective Metabolism and ISlet Cell Evaluation (PROMISE) Cohort

03.23.1430.005

Presenter Last Name: **Hanley**

Although elevated total serum non-esterified fatty acids (NEFA) are a risk factor for type 2 diabetes (T2DM), the contribution of individual NEFA to T2DM is unclear. While EPA has potent anti-inflammatory and anti-lipotoxic effects, limited data exist on the association of non-esterified EPA (thought to be enzymatically regulated) with the underlying disorders of T2DM. Our aim was to study the association of non-esterified EPA with insulin resistance (IR) and pancreatic beta-cell dysfunction. Serum samples of 484 adults at-risk for T2DM in PROMISE were analyzed for EPA in the cholesteryl ester (CE), triacylglycerol (TAG), phospholipid (PL), and NEFA pools using thin-layer-chromatography with gas-liquid-chromatography coupled to flame-ionization detectors. Glucose and insulin were measured from an oral glucose tolerance test. IR was assessed using HOMA-IR and the Matsuda index (ISI), while the insulinogenic index over HOMA-IR (IGI/IR) and the Insulin Secretion Sensitivity Index 2 (ISSI-2) determined beta-cell function. Regression covariates included sex, age, ethnicity, BMI, and family history of T2DM. Non-esterified EPA was positively correlated with esterified EPA (in the PL, CE, and TAG pools; r=0.40-0.46, p<0.0001); however, esterified EPA in the TAG, CE, and PL pools were more strongly correlated with each other (r=0.70-0.81, p<0.0001). Non-esterified EPA as a percent of total NEFA (%EPA) was significantly associated with ISI (beta [95% CI]=0.45 [0.10, 0.79]) and with HOMA-IR (beta=-0.50 [-0.85, -0.15]) in the adjusted regression models. Similarly, non-esterified EPA concentration was significantly associated with ISI (beta=0.10 [0.003, 0.21]) and with HOMA-IR (beta=-0.16 [-0.26, -0.05]). While there was a significant bivariate correlation of %EPA with both IGI/IR and ISSI-2 (r=0.11-0.15, all p<0.01), there was no significant association with these outcomes (p>0.23) in adjusted regression models. These results suggest a potential role for non-esterified EPA, which may be predominantly enzymatically regulated rather than through the diet, with insulin resistance.

Prostaglandin E2 pathway in inflammation-associated cancer development 03.24.1430.001

Presenter Last Name: **Oshima**

Accumulating evidence has indicated that chronic inflammation is associated with cancer development. Epidemiological studies reported that regular use of non-steroidal anti-inflammatory drugs (NSAIDs) including aspirin reduces a risk of gastrointestinal cancer development. Target molecules of NSAIDs are cyclooxygenases (COXs). We and other groups have shown by mouse genetic studies that COX-2 and downstream product prostaglandin E2 (PGE2) play an essential role in intestinal tumorigenesis. For gastric tumorigenesis, Helicobacter pylori infection is an important risk factor, and COX-2/PGE2 pathway is induced in the infection-associated chronic gastritis tissues. To examine the role of PGE2 signaling in gastric cancer development, we constructed transgenic mice, that express COX-2, mPGES-1, and Wnt1 simultaneously in gastric epithelial cells. Expression of COX-2 and mPGES-1 induced inflammatory responses through production of PGE2. Wnt1 is a ligand for canonical Wnt signaling, which is activated in more than 50% of gastric cancer, and thus, Wnt1 expression resulted in Wnt signaling activation in gastric epithelial cells. Although Wnt activation induced only preneoplastic lesions, cooperation of Wnt signaling and the PGE2 pathway causes gastric tumor development. Using the Wnt/PGE2 mouse model, we have found that macrophage infiltration and its activation is required for tumor promotion. Moreover, macrophage-derived cytokines including TNF-α play a role in tumorigenesis through maintenance of undifferentiated status of tumor cells. Therefore, it is possible that inhibition of PGE2 pathway will be an effective preventive or therapeutic strategy against gastric cancer development through regulation of PGE2-associated inflammation.

Fat-1 transgenic mice producing n-3 PUFA prevented Helicobacter pylori-induced gastric carcinogenesis; the efficacy and novel mechanism 03.24.1430.002

Presenter Last Name: **Hahm**

Fat-1 transgenic mice producing n-3 PUFA prevented Helicobacter pylori-induced gastric carcinogenesis; the efficacy and novel mechanism Ki Baik Hahm, Eun-Hee Kim, Young-Min Han CHA University Cancer Prevention Research Center and CHA University Bundang Medical center Seongnam, Korea ω3-polyunsaturated fatty acids (ω3-PUFA), based on their anti-inflammatory and anti-oxidative actions, had been prescribed in diverse clinical conditions, but there has been no significant report whether the ω3-PUFAs can prevent Helicobacter pylori (H. pylori)-associated precancerous lesions including

chronic atrophic gastritis and gastric cancer. Using fat-1 transgenic mice, generating ω3-PUFA through overexpression of 6-desaturase, the influence of ω3-PUFA on H. pylori-induced gastritis or cancer was investigated in this study. Wild-type C57BL/6 and fat-1 transgenic mice were deprived of food 24 h before inoculation of H. pylori and administered salt containing pellet diets to promote precancerous lesion and cancer. The mice were sacrificed after 16, 24, 36, and 44 weeks serially. At 16 weeks, the inflammatory cytokines as well as angiogenic factors were significantly increased in control mice, whereas these expressions were significantly attenuated in fat-1 transgenic mice. Huge tumorous and nodular changes simulating intestinal metaplasia were noted in control mice at 24 weeks and 36 weeks, whereas no apparent changes were noted in fat-1 transgenic mice. Molecular changes relevant to H. pylori-associated carcinogenesis were significantly decreased in fat-1 transgenic mice. Preserved lipid rafts were responsible for these protections of fat-1 transgenic mice against H. pylori infection, imposing cancer surveillance mechanisms including selective apoptosis, rejuvenation, and anti-tumorigenesis. Our result provided the rationale to apply ω-3 PUFAs as strategy to protect from H. pylori-associated cancer in high risk patients.

Novel statistical method using nutrients to define a food-based dietary pattern that distinguishes women with and without a short-term risk biomarker of developing breast cancer

03.24.1430.003

Presenter Last Name: **Hidaka**

Background: Epidemiologic evidence indicates dietary intake of individual nutrients influences breast cancer risk, but nutrient-centered results are inconsistent and difficult to apply. Recent work reveals patterns of dietary behavior are also predictive. Methods: We used a novel statistical approach to explore the relationship among nutrients and their food sources to explain the distribution of a short-term risk biomarker (cytologic atypia in random periareolar fine needle aspirates) among 63 premenopausal and postmenopausal women at an elevated risk of breast cancer development. The DHQ-I, the National Cancer Institute's food frequency questionnaire, measured nutrient and food intake. Results: After adjusting for energy intake, (a) higher food energy density, glycemic index, intake of added sugar and trans fat, and (b) lower intakes of alpha-tocopherol, iron, magnesium, and n-3 polyunsaturated fatty acids were associated with the presence of cytologic atypia ($p<0.05$). Each variable was correlated with categories of food. Based on the directionality of these associations, foods were categorized as "protective" or "risk-associated." Vegetables, tomatoes, fish, poultry, and nuts were identified as protective foods. Potatoes, non-whole grains, red meat, and cheese were associated with risk. Using discriminant analysis, the pattern of intake of these 9 foods described risk

status with 90% sensitivity and 89% specificity. Conclusions: Using a data-driven approach, we identified nutrients associated with the presence of a short-term risk biomarker of breast cancer to define a pattern of food intake that distinguishes women with an indicator of carcinogenesis risk. Dietary interventions aimed at respectively increasing and decreasing the consumption of the foods listed above in women known to be at elevated risk merit investigation for breast cancer prevention.

Delta-6 desaturase as a novel anti-cancer target 03.24.1430.004

Presenter Last Name: **Kang**

Recent studies have shown that a tumor-supportive microenvironment is characterized by high levels of pro-inflammatory and pro-angiogenic eicosanoids derived from the omega-6 (n-6) arachidonic acid (AA). Although the metabolic pathways (COX, LOX, and P450) that generate these n-6 AA eicosanoids have been targeted, the role of endogenous AA production in tumorigenesis remains unexplored. Delta-6 desaturase (D6D) is the rate-limiting enzyme responsible for the synthesis of n-6 AA. Increased D6D activity can lead to enhanced n-6 AA production. We have demonstrated that D6D activity is up-regulated during melanoma and lung tumor growth, and that suppressing D6D activity by either RNAi knockdown or a specific D6D inhibitor dramatically reduces tumor growth. Accordingly, the content of AA and AA-derived tumor-promoting metabolites is significantly decreased. Angiogenesis and inflammatory status are also reduced. Our human study also showed that D6D activity and AA synthesis are significantly increased in breast cancer tissue, compared to noncancerous tissue. Our findings identify D6D as a key factor for tumor growth and as a potential target for cancer therapy and prevention.

DHA alters lipid raft organization and cholesterol metabolism in cancer cells 03.24.1430.005

Presenter Last Name: **Corsetto**

Omega-3 fatty acids (ω3-FA), such as docosahaesanoic acid (DHA), were shown to attenuate growth and induce apoptosis in a variety of cancer cell lines derived from colonic, pancreatic, prostate, and breast cancers. In addition, recent findings indicate that ω3-FA act synergistically with chemotherapeutic agents and may be used to enhance radiosensitivity. However, the mechanism by which ω-3 PUFA inhibit cancer cells growth, is not yet well understood. It was suggested that these fatty acids might change the fluidity and structure of cell membrane, especially of lipid rafts. In fact the possible dismantling of lipid rafts is particularly important in cancer therapy. We propose that incorporation of ω3-FA alters the

profile of lipid composition influencing EGFR downstream signal transduction in breast cancer cells. We demonstrate, by HPLC-GC analysis, that DHA is incorporated and metabolized in breast cancer plasma membrane, especially in lipid rafts, with different specificity for the phospholipids moiety, altering their structure. Of note is the observation that only the treatment with DHA, compared with other PUFA, reduces lipid raft cholesterol and sphingomyelin content, indicating a possible change in raft organization. Furthermore DHA exposure modifies cholesterol synthesis, reducing farnesyl or geranyl moiety. Many acylated proteins directly interact with plasma membrane, especially lipid rafts, by their saturated moieties. Membrane incorporation of DHA induces alterations in the lateral localization of EGFR and a decrease of EGFR downstream signal transduction. In particular, DHA induces down-regulation in the EGFR-Ras-ERK1/2 and PI3K pathways .

Gut Microbiota and fatty acids 03.25.1430.001

Presenter Last Name: **Midtvedt**

Awaiting final submittal

Lipid hydrolysis products characteristic for breast milk increase the relative abundance of Bifidobacterium and Lactobacillus in microbiota isolated from infant fecal samples after in vitro fermentation. 03.25.1430.002

Presenter Last Name: **Bennike**

Some lipid hydrolysis products such as medium-chained free fatty acids (MC-FFA) and monoacylglycerols (MAG) possess antibacterial activity, while others, including oleic acid, have been reported to be essential for optimal growth of Lactobacillus species. Thus, changes of the FFA and MAG concentration in the distal ileum and colon can be expected to selectively modulate the composition of the gut microbiota. The effect of lipid hydrolysis products on the composition of infant gut microbiota is relevant in connection to breast milk and the design of formula milk. However, our knowledge is very limited. Therefore, we have determined the effect of different FFA, MAG and sphingosine on the composition of microbiota derived from infant fecal samples (age 2-5 months, n=9) after 24-hour anaerobic in vitro fermentations. The tested lipid mixtures were MC-FFAs (C10:0-C14:0 and MAG C12:0) and LC-FFAs (C16:0-C18:1 and MAG C16:0) with and without sphingosine, representing lipid-hydrolysis products characteristic for intestinal hydrolysis of breast milk lipids. Ion Torrent sequencing of the bacterial 16S rRNA revealed that the relative abundance of

Lactobacillus and Bifidobacterium were significantly increased in the presence of a MC-FFA mixture. For the Bifidobacterium the same effect was observed in the presence of a mixture containing LC-FFA with sphingosine. Contrary to this, the relative abundance of Enterobacteriaceae was significantly decreased. Generally, presence of MC-FFA or LC-FFA with sphingosine, indicated a tendency to shift the gut microbiota away from the Gram-negative bacteria (e.g. Veillonellaceae, Enterobacteriaceae) and towards the Gram-positive bacteria (e.g. Bifidobacterium, Lactobacillus, Streptococcus). Hence, this study shows that lipid-hydrolysis products representative for milk-fat (MC-FFA and sphingosine) have specific effects on the composition of the infant gut microbiota, which is known to have important impact on health and immune system development. Currently, these effects are being tested in vivo using germ-free mice inoculated with infant gut microbiota.

Fish oil attenuates omega-6 polyunsaturated fatty acid-induced dysbiosis and infectious colitis but impairs LPS dephosphorylation activity causing sepsis. 03.25.1430.003

Presenter Last Name: **Gibson**

Clinically, excessive ω-6 polyunsaturated fatty acid (PUFA) and inadequate ω-3 PUFA have been associated with enhanced risks for developing ulcerative colitis. In rodent models, ω-3 PUFAs have been shown to either attenuate or exacerbate colitis in different studies. We hypothesized that a high ω-6: ω-3 PUFA ratio would increase colitis susceptibility through the microbe-immunity nexus. To address this, we fed post-weaned mice diets rich in ω-6 PUFA (corn oil) and diets supplemented with ω-3 PUFA (corn oil+fish oil) for 5 weeks. We evaluated the intestinal microbiota, induced colitis with Citrobacter rodentium and followed disease progression. We found that ω-6 PUFA enriched the microbiota with Enterobacteriaceae, Segmented Filamentous Bacteria and Clostridia spp., all known to induce inflammation. During infection-induced colitis, ω-6 PUFA fed mice had exacerbated intestinal damage, immune cell infiltration, prostaglandin E2 expression and C. rodentium translocation across the intestinal mucosae. Addition of ω-3 PUFA on a high ω-6 PUFA diet, reversed inflammatory-inducing microbial blooms and enriched beneficial microbes like Lactobacillus and Bifidobacteria, reduced immune cell infiltration and impaired cytokine/chemokine induction during infection. While, ω-3 PUFA supplementation protected against severe colitis, these mice suffered greater mortality associated with sepsis-related serum factors such as LPS binding protein, IL-15 and TNF-α. These mice also demonstrated decreased expression of intestinal alkaline phosphatase and an inability to dephosphorylate LPS. Thus, the colonic microbiota is altered differentially through varying PUFA composition, conferring altered susceptibility to colitis. Overall, ω-6 PUFA enriches pro-inflammatory microbes and augments colitis; but prevents infection-induced systemic inflammation. In contrast, ω-3

PUFA supplementation reverses the effects of the ω-6 PUFA diet but impairs infection-induced responses resulting in sepsis. We conclude that as an anti-inflammatory agent, ω-3 PUFA supplementation during infection may prove detrimental when host inflammatory responses are critical for survival.

Elevated tissue omega-3 fatty acid status prevents chronic low-grade inflammation by altering gut microbiota 03.25.1430.004

Presenter Last Name: **Kaliannan**

Chronic low-grade inflammation contributes to the development of many chronic diseases and can be induced by bacterial lipopolysaccharides (LPS) from a dysbiotic gut microbiota. Many studies have attributed the beneficial effects of omega-3 (n-3) polyunsaturated fatty acids (PUFA) to their anti-inflammatory properties. However, the relationship between the gut microbiota and the anti-inflammatory effects of tissue n-3 PUFA has not been well investigated. Using both a transgenic mouse model producing n-3 from n-6 PUFA and dietary supplementation with n-3 PUFA, here we show that increased n-3 PUFA status with reduction of the n-6/n-3 PUFA ratio in the tissues of aged mice leads to dramatic changes in the gut microbiota – a decrease in LPS-producing bacteria with an increase in LPS-suppressing bacteria of the stool and ileum, associated with greater expression of anti-microbial peptides such as intestinal alkaline phosphatase (IAP) and Reg3γ in the ileum. Importantly, the changes in gut microbiota were also associated with a significant reduction in LPS, LPS-related pathway components, and gut permeability, as well as a concomitant decrease in inflammatory cytokines (e.g., TNF-α, IL-1β, and IL-6) and other inflammatory factors (e.g., NF-kB, iNOS, and COX-2). Our findings reveal a novel interaction between the host tissue essential fatty acid status and the gut microbiota, and provide a new mechanism by which n-3 PUFA suppress chronic low-grade inflammation via modulation of the gut microbiota. Kanakaraju Kaliannan, Bin Wang, Xiangyong Li, Jing X. Kang Laboratory of Lipid Medicine and Technology, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA

Milk lipids in infant formulas modifies the proteolysis, microbiota and intestinal physiology in neonatal piglets 03.25.1430.005

Presenter Last Name: **Le Huerou-Luron**

Human milk is generally recognized as the gold stallion in neonatal nutrition. Structure, composition and physiological properties of Human milk are better

mimicked in infant formulas that include milk lipids and milk fat membrane extracts although very few infant formulae use milk lipids more expensive than vegetable lipids. Two formulas based on i) vegetable fat (STD) or ii) a blend of vegetable fat and milk lipids stabilized by milk fat membrane extracts (EXP) were distributed with an automatic milk feeder to piglets from birth until 28 days. After euthanasia, Intestinal contents and tissues (proximal jejunum and ileum) and mesenteric lymph nodes (MLN) were collected. The residual immunoreactivity of β-lactoglobulin (β-lg) and caseins (Cns) present in the digestive compartments was determined by ELISA. The paracellular permeability (using FITC-dextran 4000) was evaluated using Ussing chambers. The EXP formula enhanced the resistance to proteolysis of β-lg and Cns. It modified microbiota with an increase in proteobacteria and a decrease in firmicutes. Ileal density (g/cm) was greater in EXP-fed piglets at 28 d. A decreased jejunal permeability was observed between d7 and d28 in EXP-fed piglets which was not observed with STD. There was an important effect of formula components on the secretory activity of MLN: a major immunosuppressor effect of the lipid fraction extracted from digestas of both formulas was evidenced. In conclusion, the lipid composition in infant formulas influenced the neonatal intestinal physiology through release of immunomodulatory lipids, modulation of proteolysis and modification of progressive colonisation of the infant digestive tract by bacteria.
