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POSTER ABSTRACTS

For all poster abstracts:

Presenting author is underlined

indicates equal effort contributed by first authors

** indicates lightning talk selection*

1. The significance of lipids in chronic obstructive pulmonary disease *

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Background: Chronic obstructive pulmonary disease (COPD) is currently the third leading cause of death in the United States and typically affects cigarette smokers; no cure is available and treatment is limited. To improve our understanding of COPD, perturbed pathways in human COPD subjects were compared to those from a smoking mouse model of COPD. Our aim was to identify dysregulated pathways associated with disease with the long term goal of identifying markers and potential treatments. **Methods:** Plasma was collected from current and former COPD smokers (n=131) with >10 pack-years smoking history, and from C57BL/6 mice (n=126) exposed to ambient air or cigarette smoke. Protein precipitation and liquid-liquid extraction of plasma resulted in lipid and aqueous compounds which underwent LC/MS-based metabolomics. Controls and smoking mice were compared using t-tests. Human samples were analyzed in R using regression models with covariate adjustment for age, gender, BMI, and smoking status. **Results:** Comparison of smoking and control mice identified statistically significant metabolites ($p \leq 0.05$) belonging to pathways such as glycerophospholipid metabolism ($p=2.04e^{-7}$), glycosylphosphatidylinositol-anchor biosynthesis ($p=1.07e^{-4}$), regulation of autophagy ($p=3.55e^{-4}$), phosphatidylinositol signaling system ($p=5.33e^{-4}$), inositol phosphate metabolism ($p=0.0232$), glycerolipid metabolism ($p=8.86e^{-4}$), and purine metabolism ($p=0.0176$). Analysis of COPD subjects identified significant metabolites ($p \leq 0.05$) which were mapped to glycerophospholipid metabolism ($p=1.45e^{-11}$), sphingolipid metabolism ($p=3.20e^{-11}$), phosphatidylinositol signaling system ($p=1.14e^{-4}$), glycerolipid metabolism ($p=2.25e^{-4}$), glycosylphosphatidylinositol-anchor biosynthesis ($p=2.76e^{-4}$), regulation of autophagy ($p=9.09e^{-4}$), and inositol phosphate metabolism ($p=0.05$). **Conclusion:** Metabolomics profiling revealed significant overlap in perturbed lipid pathways in both human subjects and our COPD mouse model. These pathways are associated with oxidative stress, toxicity of pulmonary irritants, inflammation, lipid and cell signaling, cell death, division, differentiation, and immune system and defense. This suggests that multiple mechanisms are at play in COPD and can be interrogated further using our mouse model.

2. Cysteinyl leukotriene 2 receptor enhances angiogenesis, vascular permeability and tumor metastasis.*

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Excessive or insufficient angiogenesis can lead to number of diseases including atherosclerosis, diabetic retinopathy and cancer. Although long-standing inflammation secondary to chronic infection or irritation mediated by immune cells has been implicated in cancer progression, the contribution of endothelial cells (EC) in the generation of inflammatory mediators and their effects on angiogenesis is relatively unknown. One of the pro-inflammatory mediators produced by EC are cysteinyl leukotrienes (LTC₄, LTD₄ and LTE₄), and they mediate their effects through two main receptors, CysLT₁R and CysLT₂R. Cys-LTs have been shown to be involved in several human cancers including breast cancer and melanoma. Tumor microenvironment comprises numerous signaling molecules and pathways that influence angiogenic response leading to increased and aberrant vascularization. Further, angiogenesis is required for the supply of nutrients and oxygen for tumor growth and also serves as a route of tumor cells metastasis. Therefore, we investigated the role of CysLTR in angiogenesis, tumor progression and metastasis, using CysLT₁RKO and CysLT₂RKO mice. We observed enhanced CysLT₂R expression, and not CysLT₁R in wild type (WT) tumors and tumor growth as well as angiogenesis were significantly decreased in CysLT₂RKO mice compared to WT and CysLT₁RKO mice in LLC tumor model. Further, though few in number, tumor vessels in CysLT₂RKO mice showed intact pericyte coverage and reduced permeability with simultaneous reduction in tumor cell metastasis to the lung. Furthermore, we found that activation of CysLT₂R, but not CysLT₁R increased EC contraction leading to junctional destabilization and permeability in vitro. Importantly, our results show that CysLT₂R activates Rho kinase and inhibition of Rho kinase significantly attenuated CysLT₂R-induced EC contraction and permeability. Taken together, our results suggest that CysLT₂R promotes leaky blood vessel formation, enhancing tumor

growth and metastasis to the lung. Blocking CysLT₂R could offer novel CysLT₂R-targeted, therapeutic candidates for the treatment of cancer and other angiogenic disorders.

3. PLA2G5-expressing M2 macrophages in mouse and human type 2 inflammation *

Munehiro Yamaguchi, Jennifer Zacharia, Tanya M. Laidlaw, [Barbara Balestrieri](#)

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Phospholipases A₂ (PLA₂) are enzymes that liberate membrane bound lipids in a tissue and cell-specific fashion. We have previously shown that group V secretory PLA₂ (Pla2g5) is induced by IL-4 in mouse and human M2 macrophages. We also showed that Pla2g5 is required for the development of M2 macrophages and their effector functions in a mouse model of type 2 allergic airway inflammation. However, the function of PLA2G5 in human M2 activation and type 2 inflammation was ill-defined. Transglutaminase 2 (TGM2), a protein crosslinking enzyme, is a newly identified marker of both human and mouse IL-4-activated M2 macrophages, and is also found in the lungs of patients with asthma. Here we report that PLA2G5 and TGM2 colocalized in macrophages of human nasal polyp tissue obtained from patients with type 2 eosinophilic inflammation, and their co-expression positively correlated with the number of eosinophils in each tissue specimen. We demonstrate that in human monocyte-derived macrophages activated by IL-4, PLA2G5 translocated and colocalized with TGM2 in the cytoplasm and on the membrane of macrophages. Moreover, knocking down PLA2G5 with siRNA reduced macrophage transglutaminase activity, while mass spectrometry analysis of lipids also showed reduced prostaglandin E₂ (PGE₂) production. Finally, exogenous PGE₂ restored transglutaminase activity of PLA2G5-siRNA-treated macrophages. Thus, our study shows a novel function of PLA2G5 in regulating the transglutaminase activity of human IL-4-activated M2 macrophages through PGE₂ generation and suggests that PLA2G5 is a functionally relevant enzyme that may have therapeutic value for the treatment of human Th2 inflammatory disorders.

4. Novel omega-3 endocannabinoid epoxides emanating from the cross-talk between the endocannabinoid and cytochrome P450 metabolic pathways *

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The human body contains endocannabinoids that elicit similar effects as Δ9-tetrahydrocannabinol (THC), the principal component of cannabis, which produces similar psychoactive and anti-nociceptive effects. The two most well studied endocannabinoids include anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which are derived from the omega-6 arachidonic acid (AA). Previously, we showed that both of these omega-6 endocannabinoids are substrates for metabolism by epoxygenases to form novel AEA and 2-AG epoxides with distinct biological activity. Recently, it was discovered that the omega-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) also form endocannabinoids eicosapentaenoic ethanolamide (EPEA) and docosahexaenoic ethanolamide (DHEA), respectively. Using LC-MS/MS we demonstrate that EPEA and DHEA are metabolized by the epoxygenases to produce novel EPEA and DHEA epoxides with unknown biological functions. We show that we can detect these metabolites endogenously in the brain and other peripheral organs in pigs and rats at the same level as AEA. Furthermore we show that the epoxygenases in the tissues are capable of producing these metabolites. When specific epoxygenases in the brain and vasculature were screened for the metabolism of these substrates, we identified CYP2J2 as the primary metabolizer of EPEA and DHEA. Furthermore we elucidate the nuances of ligand-protein interactions using biophysical methods. We evaluated the biological effects of these newly discovered molecules in the following assays – neuro-inflammatory assays, cannabinoid receptor binding and activity assay, vasodilation using porcine coronary artery, platelet aggregation assay and angiogenesis study using human microvascular endothelial cells (HMVEC). We found that these newly discovered molecules are anti-inflammatory, vasodilatory, anti-platelet aggregatory and anti-angiogenesis. In summary, we have discovered novel omega-3 endocannabinoid epoxides that arise from the cross-talk of the enzymes in the endocannabinoid and epoxygenases pathway that elicit distinct biological activity.

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5. Increased plasma levels of select deoxy-ceramide and ceramide species are associated with increased odds of diabetic neuropathy in type 1 diabetes: A pilot study *

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Plasma deoxy-sphingoid bases are elevated in type 2 diabetes patients and correlate with the stage of diabetic distal sensorimotor polyneuropathy; however, associations between deoxy-sphingolipids (DSL) and neuropathy in type 1 diabetes have not been examined. Using mass spectroscopy, plasma levels of DSL and free amino acids in DCCT/EDIC type 1 diabetes participants (n=80), with and without symptoms of neuropathy were investigated. Patient-determined neuropathy was based on 15-item self-administered questionnaire [Michigan Neuropathy Screening Instrument] developed to assess distal symmetrical peripheral neuropathy in diabetes. Patients who scored ≥ 4 , or reported inability to sense their feet during walking or to distinguish hot from cold water while bathing were considered neuropathic. Plasma levels of ceramide, sphingomyelin, hexosyl- and lactosylceramide species, and amino acids were measured and analyzed relative to neuropathy status in the patient. Deoxy-C24-ceramide, C24- and C26-ceramide were higher in patients with neuropathy than those without neuropathy. Cysteine was higher in patients with neuropathy. No differences in other sphingolipids or amino acids were detected. The covariate-adjusted Odds Ratios of positive patient-reported neuropathy was associated with increased levels of deoxy-C24-, and deoxy-C24:1-ceramide; C22-, C24-, and C26-ceramide; and cysteine. Thus, plasma deoxy-ceramide and ceramide species may have diagnostic and prognostic significance in diabetic neuropathy.

6. Biosynthesis and quantification of hemiketal eicosanoids formed via cross-over of 5-LOX and COX-2 activities *

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Leukotriene and prostaglandin synthesis starts from the common substrate, arachidonic acid (AA), oxygenated by 5-lipoxygenase (5-LOX) and cyclooxygenase 2 (COX-2), respectively. Although these two pathways have been considered separate biosynthetic pathways, we have shown recently that 5-LOX and COX-2 can act in tandem, leading to the synthesis of the lipid mediators hemiketals (HK) E₂ and D₂. Thus, oxygenation of AA by 5-LOX produces 5-hydroxyeicosatetraenoic acid (5-HETE) that can be consecutively oxygenated by COX-2 producing an unstable diendoperoxide, which in turn, rearranges into HKD₂ and HKE₂. We surmise that biosynthesis of HKs involves the exchange of 5-HETE from 5-LOX to COX-2 expressing cells. However, it is currently unclear how these cells regulate the production of HKs. Hence, in this study, we investigated the time-course of production of HKs in human leukocytes exposed to LPS (10 μ g/mL) and calcium ionophore (A23187; 5 μ M) in order to stimulate COX-2 and 5-LOX activity, respectively. The simultaneous analysis of COX-2 and 5-LOX eicosanoids (PGE₂, 5-HETE, LTB₄, HKD₂, HKE₂) was performed using LC-MS/MS. To increase sensitivity, samples were derivatized by converting the carboxylic acid moiety to a stable cationic N-(4-amino-methylphenyl)pyridinium-amide (AMPP derivatization). Specific inhibition of COX-2 (NS398) and 5-LOX activity (AA861 and MK-886) resulted in reduced formation of HKs. The production of 5-LOX products, including 5-HETE and LTB₄, peaked at early time points (15 min) and decreased at later time points (48 h). Similar to the 5-LOX products, HKE₂ and HKD₂ also exhibited the highest values at 15 min. Conversely, PGE₂ level was lower at shorter incubation times and increased in a time dependent manner. Furthermore, the 5-LOX and COX-2 inhibitors reduced or abolished the production of HKs indicating that these two enzymes are essential in the synthesis of HKs. Therefore, our results show that 5-LOX and COX-2 are essential in the synthesis of HKs.

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7. A conserved circular network of coregulated lipids modulates innate immune responses *

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Pathogen-recognition by Toll-like receptors (TLRs) and the subsequent responses involve distinct membrane-dependent processes including receptor trafficking and signaling, cytokine secretion, and cell polarization. Here, we combined a systematic genetic perturbation strategy of sphingolipid metabolism with quantitative lipidomics, and a functional characterization of TLR-related processes across different stimuli. Integrative analysis revealed sphingolipid metabolism to cooperate in distinct feedback loops, enabling the cell to enhance or dampen pan-TLR signaling or prioritize between plasma membrane and endosomal TLR pathways. Perturbing sphingolipid metabolism affected the entire membrane lipid landscape, revealing an evolutionary conserved circular network or coregulated lipids reflecting metabolic pathways, adaptation strategies, lipid abundance, and subcellular localization. Integration of the diverse TLR-induced inflammatory phenotypes with changes in lipid abundance assigned distinct functional roles to individual lipid species organized across the network. This annotation accurately predicted the inflammatory response of cells derived from patients suffering from lipid storage disorders, based solely on their altered membrane lipid composition. The data-driven approach described here empowers the understanding of higher-level organization of membrane lipid function in diverse biological systems and starts the unbiased annotation of lipid function using a method broadly applicable to other metabolic pathways.

Keywords: Toll-like receptor, lipid metabolism, functional lipidomics

8. UPLC-MS/MS identifies lipids associated with perceptual speed performance in healthy adults *

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Introduction: Several studies have demonstrated associations between plasma lipids and neurological diseases, including Alzheimer's disease, Parkinson's disease and schizophrenia. However, the relationship between circulating lipids and cognition in healthy individuals has not been well defined. We determined associations between circulating plasma lipids and cognitive measurements in a healthy older population.

Methods: Healthy individuals (age range 43-84, n=286), completed a comprehensive neurocognitive test battery including digit symbol coding (DSC), a measure of perceptual speed performance. Lipids were extracted from 10 μ L plasma and lipidomic analysis performed using UPLC-MS/MS with a solvent system consisting of water/methanol/tetrahydrofuran containing 10mM ammonium formate. Fatty chain identification of phospholipid peaks were performed on pooled healthy control samples using lithium adducts. **Results:** We were able to measure 381 lipid species and assign fatty acid composition for the majority. Linear regression analysis (adjusting for age, gender, BMI, total cholesterol, HDL, triglycerides and statin use) identified lipid species associated with DSC, with 64 of the 381 measured lipids having a significant association ($p < 0.05$, uncorrected). 24 lipids remained significant after correction using the Benjamini-Hochberg method ($p < 0.05$). Our analysis identified diacyl and ether phospholipids containing omega-3 fatty acids (20:5, 22:5 or 22:6) as positively associated with DSC score. **Discussion and Conclusion:** Digit symbol coding is a sensitive measure of perceptual speed and is included in many standard measures of intelligence. Performance deficits of DSC represents one of the largest effect size findings in schizophrenia neuropsychological literature. Several groups have identified plasma lipid changes associated with schizophrenia similar to those observed with DSC in healthy individuals in this study. This suggests that plasma lipid changes seen in schizophrenia may, in part, be reflective of perceptual speed changes and that esterified omega-3 fatty acids could be important in the neurological changes associated with the disease.

9. Metabolic perturbations of postnatal growth restriction and hyperoxia-induced neonatal pulmonary hypertension in a rat model of bronchopulmonary dysplasia *

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Pulmonary hypertension (PH) is a common manifestation of bronchopulmonary dysplasia (BPD) and contributes to increased morbidity and mortality of preterm birth. Postnatal growth restriction has emerged as an independent contributor to the development of PH. Utilizing a rat model combining hyperoxia and post-natal growth restriction, our lab recently investigated the lung histological and protein expression changes caused by BPD-associated vasculature remodeling and consequent PH. The study presented herein utilized a multi-platform approach consisting of complex lipids and lipid mediators, as well as primary metabolism, to characterize the metabolome of lung tissue and plasma from this rodent model. Specifically, Sprague-Dawley neonates underwent three models of PH (growth-restriction, hyperoxia or combined) relative to control. Univariate analyses, multivariate modeling and biochemical networks were utilized to identify and visualize metabolic perturbations. Metabolic changes were characterized by lung-specific 1) decreased total plasmalogen phosphatidylcholines and increased arachidonic acid and docosahexaenoic acid that suggest increased phospholipase A2 activity, linked to surfactant alteration, 2) increased total (non-esterified and esterified) cytochrome P450 (CYP) oxylipins and decreased 12/15-LOX oxylipins were characteristic of pulmonary vascular remodeling, and 3) alterations in primary metabolism indicate a shift to fatty acid oxidation and mitochondrial respiration, as well as pathways indicative of reactive oxygen species production. Although plasma metabolite changes generally showed similar trends as the lung, there were differences. Numerous circulating fatty acids were elevated while total phosphatidylcholines were decreased. Furthermore, plasma non-esterified 5-LOX and soluble epoxide hydrolase (sEH) products involved in the regulation of inflammation, vascular tone, and immune response during development were increased. The present study identifies unique and conserved metabolic perturbations which accompany growth-restriction or hyperoxia-induced PH, providing unique insight into disease pathophysiology.

10. Dietary linoleic acid excess plays a key role in the development of obesity – a multiomics-based analysis *

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Although dietary fat excess is an established cause of obesity in animal models and humans, the molecular link between fat intake and energy dysregulation has not been fully addressed. We have micro-dissected hypothalamic nuclei, energy centers in the brain, from mice fed high fat diet (HFD) or control diet (CD) for 3days, 2, 6, and 16weeks and subjected to transcriptome (RNA-Seq) and Lipidome (LC/MS) analyses. Accumulation of n-6 (linoleic and arachidonic) acylesters evident already at 3days of HFD and increased thereafter, has prompted us to hypothesize that linoleic acid be the cause of obesity. Mice fed HFD regimen rich in linoleic acid (18%kcal) gained more weight than those fed low linoleic acid diet (1.2%kcal). Weight gain by high linoleic acid diet was accompanied by (1) increased hypothalamic PGE₂ content, (2) signs of hypothalamic inflammation, (3) attenuated hypothalamic pStat3 response to anorexigenic hormone, leptin, (4) decreased systemic fat oxidation, (5) adipose tissue inflammation, and (6) insulin resistance. Correlation coefficients were calculated between all possible combinations of lipid molecules and transcripts over four time points and three hypothalamic nuclei. Larger number of significant correlations with gene expression were found for n-6 unsaturated than saturated acylesters, suggesting more tight epistatic interactions between n-6 fatty acids and gene regulation. Taken together, our data show a causal role of dietary linoleic acid in the development of obesity in mice, possibly through a resultant accumulation of n-6 acylesters within the hypothalamic energy centers.

11. Allosteric regulation of phospholipase A₂ by membranes *

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Defining the molecular details and consequences of the association of water-soluble proteins with membranes is fundamental to understanding protein–lipid interactions and membrane functioning.¹ Phospholipase A₂ (PLA₂) enzymes, which catalyze the hydrolysis of phospholipid substrates that comprise the membrane bilayer, provide the ideal system for studying protein–lipid interactions.² Our current study focuses on understanding the catalytic cycle of two different recombinant human intracellular PLA₂s: the GIVA cPLA₂ and GVIA iPLA₂, which are responsible for arachidonic acid release for eicosanoid signaling and for membrane phospholipid remodeling, respectively. Molecular dynamics (MD) simulations, guided by hydrogen/deuterium exchange mass spectrometric (DXMS)³ experimental data, were used to show that the channel to the active sites of these PLA₂s are opened upon allosteric interaction of the enzyme surface with the membrane to facilitate entry of the substrate phospholipid. This constitutes the first detailed study describing the binding and the interaction mechanism of intracellular PLA₂s with the membrane bilayer as well as how they bind a single phospholipid molecule in the catalytic site. These enzymes are implicated in many diseases, and understanding their detailed mechanism of action will aid in the discovery of new therapeutics.

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12. De novo synthesis of branched chain fatty acids links amino acid, carbohydrate and lipid metabolism in white and brown adipocytes *

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The importance of *de novo* lipogenesis (*DNL*) is gaining increased attention as studies have shown its importance in insulin sensitivity and vascular homeostasis. While it is generally presumed that palmitate is the primary product of *DNL* in mammalian cells, through ¹³C stable isotope tracing, we have observed that cultured brown and white adipocytes synthesize a broader range of fatty acids. Here we show that adipocytes utilize carbon not just from acetyl CoA pools but also CoA intermediates of the branched chain amino acid (BCAA) catabolic pathway leading to production of monomethyl branched and odd chain fatty acids (mmBCFA and OCFA). In addition, provision of propionate or short branched chain fatty acids such as isovalerate to 3T1L1 adipocytes leads to increased mmBCFA and OCFA levels while decreasing palmitate abundance. This indicates how altered substrate availability or amino acid breakdown modulates the fatty acid composition of the cell. It has been speculated that *in vivo*, mmBCFAs and OCFAs are primarily derived from the diet however using D₂O labeling we have demonstrated that *de novo* synthesis of these fatty acids occurs *in vivo* and is significantly reduced upon high fat feeding. Decreased adipose BCAA catabolism is associated with obesity and increased BCFA levels in human adipose tissue have previously been shown to positively correlate with insulin sensitivity indicating BCFAs may play an important role in normal adipose function. Our findings, characterizing the drivers of the *de novo* synthesis of mmBCFAs are an important step in determining the biological function of these fatty acids which may provide a unique metabolic signal linking amino acid, carbohydrate and lipid metabolism.

13. Application of lipidomics for identifying novel biomarkers of a chronic multisymptom illness affecting the 1991 Gulf War veterans.

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Introduction: Gulf War Illness (GWI) affects nearly 25% of the 700,000 veterans from the 1991 Gulf War and presents with a cluster of symptoms ranging from memory impairment, motor problems, fatigue and gastrointestinal problems. Due to the complexity of the clinical presentation and limited understanding of the pathophysiology of GWI, this illness remains difficult to diagnose. Furthermore, the current GWI diagnostic procedures rely heavily on the self-report of exposures and symptoms. The goal of this study is apply lipidomics technology in order to identify blood-based biomarkers that can provide an objective diagnosis of GWI. **Methods:** Plasma lipid extracts from age and gender matched GW veterans with GWI and controls were subjected to hydrophilic interaction liquid chromatography (HILIC) and mass spectrometry (MS) analysis in the Fourier Transform Mass Spectrometry (FTMS) mode at 30,000 resolution on the Thermo LTQ-Orbitrap mass spectrometer. Lipid extracts were also analyzed using HILIC LC/MS with Thermo LTQ mass spectrometer to separate phospholipid (PL) classes and individual species were identified and quantified with the LipidomeDB software. **Results:** Our findings suggest that compared to control GW veterans, those with GWI have elevated levels of ether phosphatidylcholine and lyso-platelet activating factor (lyso-PAF). Several individual PL species that contained omega-3 and omega-6 fatty acids were also increased in veterans with GWI compared to controls. However, long-chain acylcarnitine species were decreased in GWI compared to controls. **Conclusion:** Our findings suggest that an evaluation of blood PL and mitochondrial lipids may lead to the development of biomarkers for assisting clinicians with diagnosing GWI. Changes in these lipids could be due to the observed aberrant activation of the immune/inflammatory pathways and potential failure of mitochondria function, which warrant further investigation.

14. Inflammatory mediator profiling in sweat: A potential noninvasive diagnostic for inflammatory skin diseases

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Sweat is a complex biological fluid with potential diagnostic value for the investigation of skin disorders. Previous efforts in sweat testing focused on analysis of small molecules and ions for forensic and diagnostic testing, but with advances in analytical and sweat collection techniques, there has been recent interest in conducting metabolomic analyses of sweat to establish biomarkers for and understand mechanisms of skin inflammation and repair. Our study aims to characterize the lipid mediator profile in sweat and identify differences in these profiles between subjects with and without atopic dermatitis. Using the Macroduct[®] collection device, originally developed for cystic fibrosis diagnostic testing of sweat chloride in neonates, sweat (40-100 µL) was collected from subjects with and without atopic dermatitis (n = 12 per group), and profiled over 100 lipid mediators including oxylipins, endocannabinoids and ceramides/sphingoid bases using liquid chromatography-tandem mass spectrometry. A total of 61 lipid mediators including 39 oxylipins, 13 endocannabinoids and 9 ceramides/ sphingoid bases were detected in the sweat. Increases in concentrations of linoleate-derived diols and triols, and C30-C40 [NS] ceramides were observed in the sweat of subjects with atopic dermatitis (p < 0.05, two-tailed Student's t-test). Separation of the subject groups was possible using partial least squares-discriminant analysis with separation primarily due to increased concentrations of [NS]-type ceramides in the sweat of subjects with atopic dermatitis. Our current findings demonstrate the presence of lipid mediators in sweat, and suggest differences in the lipid mediator profile between subjects with and without atopic dermatitis. Sweat mediator profiling therefore may provide a non-invasive assessment of atopic dermatitis pathogenesis and mechanistic progression, and aid in novel target elucidation and assessment of therapeutic efficacy.

15. Alterations in lysophosphatidylcholine-related metabolic parameters in the plasma of mice with bacterial peritonitis

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Lysophosphatidylcholine (LPC) concentration is decreased in the plasma of septic patients compared with healthy group. Further, administration of LPC to septic mice has protective effects against sepsis. So the alteration of LPC metabolism may be important for understanding pathophysiology of sepsis. However, the mechanisms of sepsis-induced decrease in plasma LPC levels are not currently well known. In this study, we examined alterations of LPC-related metabolic parameters in mice with cecal ligation and puncture (CLP), a model of sepsis regarded as clinically reliable. We investigated alterations of phosphatidylcholine (PC), lysophosphatidic acid (LPA), secretory phospholipase A2 (sPLA2), lecithin: cholesterol acyltransferase (LCAT), autotaxin (ATX) and albumin in the plasma of CLP-treated mice. LPC, PC and albumin levels were decreased in the plasma of CLP-treated mice compared with control group, and enzyme activities of LCAT, ATX and sPLA2 were also decreased. On the other hand, LPA measured with ELISA was increased in the plasma of CLP-treated mice. As LPA is a well-known key molecule in inflammation, the alteration of plasma LPA in CLP-treated mice could affect the immune system in sepsis. We could not find causal association between ATX, LCAT and plasma LPC level, as ATX inhibitor (PF-8380) did not affect plasma LPA or LPC in CLP-treated mice. Further study is needed to elucidate the exact mechanisms about alterations of LPC metabolism in CLP-treated mice.

Key word: sepsis, cecal ligation and puncture, plasma, lysophosphatidylcholine, lysophosphatidic acid, metabolism

16. The role of dipalmitoylphosphatidylcholine produced by lysophosphatidylcholine acyltransferase 1 in polyunsaturated fatty acid-induced cytotoxicity

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The degree of fatty acid unsaturation in membrane phospholipids affects many membrane-associated functions and can be influenced by dietary fatty acids and by altered activities of lipid-metabolizing enzymes. Therefore, the fatty acid composition in membrane phospholipids must be tightly regulated. When loaded with excess saturated fatty acids (SFAs), cells convert SFAs to unsaturated fatty acids (UFAs) to prevent the incorporation of excess SFAs into membrane phospholipids. However, it is unclear how cells deal with the incorporation of excess UFAs because UFAs cannot be converted into fatty acids with fewer double bonds. Here, we show that loading mammalian cells with polyunsaturated fatty acids (PUFAs) stimulates the production of dipalmitoylphosphatidylcholine (DPPC) to protect against PUFA-induced cytotoxicity. DPPC was produced time- and dose- dependently by PUFA treatment and its production was correlated with the production of PUFA-containing phospholipids. An RNAi screen of lipid-metabolizing enzymes revealed that lysophosphatidylcholine acyltransferase 1 (LPCAT1) was involved in the DPPC production. Moreover, prevention of DPPC production by LPCAT1 knockdown markedly enhanced the cytotoxicity and unfolded protein response (UPR) induced by loading with excess PUFAs. PUFA-induced cytotoxicity was dependent on caspase and UPR sensor proteins inositol requiring 1 (IRE1) and protein kinase R-like endoplasmic reticulum kinase (PERK), indicating that excess PUFAs trigger UPR-mediated apoptosis. In murine retina, in which PUFAs are highly enriched, DPPC was produced along with increases of PUFA-containing phospholipids. In LPCAT1 knockout mice, DPPC level was reduced and UPR was activated in the retina. Our results provide insight to understanding of the retinal degeneration seen in *rd11* mice that lack LPCAT1.

17. Human 15-Lox-1 active site mutations alter inhibitor binding and decrease potency

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Human 15-Lipoxygenase-1 (h15-LOX-1 or h12/15-LOX) reacts with polyunsaturated fatty acids and produces bioactive lipid derivatives that are implicated in many important human diseases. One such disease is stroke, which is the fifth leading cause of death and the first leading cause of disability in America. The discovery of h15-LOX-1 inhibitors could potentially be novel therapeutics in the treatment of stroke, however, little is known about the inhibitor/active site interaction. This study utilizes site-directed mutagenesis, as well as molecular modeling, to gain a better structural understanding of inhibitor interactions with the active site. We have generated eight mutants (R402L, R404L, F414I, F414W, E356Q, Q547L, L407A, I417A) of h15-LOX-1 to determine whether these active site residues interact with our two structurally similar h15-LOX-1 inhibitors, a **ML094** derivative and **ML351**. IC₅₀ values and steady-state inhibition kinetics were determined with the eight mutants and four of the mutants affected inhibitor potency relative to wild type h15-LOX-1 (F414I, F414W, E356Q and L407A). The data indicate that **ML094** and **ML351** bind to similar sites in the active site but have subtle differences in their binding modes.

18. PUFA-mediated proinflammatory response to ionizing radiation

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The identification of cancer patients who might develop severe adverse reactions in response to radiotherapy has been hindered by the complexity of individual variation in sensitivity to radiation. The molecular response to ionizing radiation, however, is still not completely understood. Here we screened mouse serum for metabolic alterations following an acute exposure to gamma radiation using a multi-platform, mass-spectrometry-based strategy. A global, molecular profiling allowed to monitor the effects of radiation exposure on key biochemical pathways. Exposure to gamma radiation induced a significant increase in the serum levels of ether phosphatidylcholines (PCs) while decreasing the levels of diacyl PCs carrying PUFAs. In exposed mice, levels of pro-inflammatory, oxygenated metabolites of arachidonic acid increased, whereas levels of anti-inflammatory metabolites of omega-3 PUFAs decreased. The obtained molecular biosignature might be used as an indicator of radiation exposure and, potentially, as a predictor of radiosensitivity. Verification studies are currently undergoing in human samples. If validated, baseline levels of eicosanoids (e.g., omega-6/omega-3 ratio) might serve as a companion diagnostic tool for radiation therapy, to help differentiate cancer patients who would respond best to radiotherapy treatment from radiosensitive patients, who may be unable to tolerate the additional inflammatory response induced by radiotherapy. Most importantly, the ability to control eicosanoids pathways with pharmacological or dietary interventions (i.e., omega-3 supplementation) might alleviate and eventually offset many of the side effects linked to radiation therapy.

19. Simultaneous quantitative profiling of 20 isoprostanoids from omega-3 and omega-6 polyunsaturated fatty acids by LC-MS/MS in various biological samples.

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Isoprostanoids are a group of non-enzymatic oxygenated metabolites of polyunsaturated fatty acids. It belongs to oxylipins group, which are important lipid mediators in biological processes, such as tissue repair, blood clotting, blood vessel permeability, inflammation, and immunity regulation. Recently, isoprostanoids from eicosapentaenoic, docosahexaenoic, adrenic and α -linolenic namely F₃-isoprostanes, F₄-neuroprostanes, F₂-dihomo-isoprostanes and F₁-phytoprostanes, respectively have attracted attention because of their putative contribution to health. Since isoprostanoids are derived from different substrate of PUFAs and can have similar or opposing biological consequences, a total isoprostanoids profile is essential to understand the overall effect in the testing model. However, the concentration of most isoprostanoids range from picogram to nanogram, therefore a sensitive method to quantify 20 isoprostanoids simultaneously was formulated and measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The lipid portion from various biological samples was extracted prior to LC-MS/MS evaluation. For all the isoprostanoids LOD and LOQ, and the method was validated on plasma samples for matrix effect, yield of extraction and reproducibility were determined. The methodology was further tested for the isoprostanoids profiles in brain and liver of LDLR^{-/-} mice with and without docosahexaenoic acid (DHA) supplementation. Our analysis showed similar levels of total F₂-isoprostanes and F₄-neuroprostanes in the liver and brain of non-supplemented LDLR^{-/-} mice. The distribution of different F₂-isoprostane isomers varied between tissues but not for F₄-neuroprostanes which were predominated by the 4(RS)-4-F₄-neuroprostane isomer. DHA supplementation to LDLR^{-/-} mice concomitantly increased total F₄-neuroprostanes levels compared to F₂-isoprostanes but this effect was more pronounced in the liver than brain.

20. Sphingosine 1-phosphate bound to ApoM+HDL modulates generation of the adaptive immune response at different stages of lymphocyte ontogeny

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Activation and mobilization of lymphocytes is a tightly controlled process, the dysregulation of which can have severe pathophysiological consequences. The lipid mediator sphingosine 1-phosphate (S1P) has well-characterized roles in numerous facets of endothelial cell biology; however, lymphocyte-intrinsic effects of S1P signaling, beyond modulation of trafficking, have only recently gained attention. Although the majority (~65%) of plasma S1P is bound to apolipoprotein M (ApoM) in the high-density lipoprotein (HDL) particle, how the ApoM-S1P complex regulates immunity is unknown. Here we show that, while dispensable for trafficking, ApoM-S1P restrains lymphopoiesis by activating the receptor S1P₁ on bone marrow lymphocyte progenitors. *Apom*^{-/-} mice have increased Lin⁻Sca1⁺cKit⁺ hematopoietic stem and progenitor cells (LSK) and common lymphoid progenitors (CLP) in BM. Upon immunization with immunogenic peptide, *Apom*^{-/-} mice develop more severe experimental autoimmune encephalomyelitis (EAE), characterized by increased lymphocytes, particularly T_h1 T cells, in the central nervous system and breakdown of the blood-brain barrier. However, *in vitro* studies indicate that ApoM⁺HDL does not regulate proliferation of naïve mature lymphocytes, but rather modulates T_h1 versus T_h17 phenotype decisions. Lipidomic analyses of plasma revealed loss of ApoM affects concentrations of multiple sphingolipid species, which may also impact development of adaptive immune responses. These data demonstrate that the ApoM-S1P-S1P₁ signaling axis regulates the lymphocyte compartment at various stages of ontogeny, from progenitor to mature cell, by differentially affecting proliferation or phenotype. Thus, S1P chaperones maybe provide developmental stage-specific novel targets for the modulation of adaptive immunity.

21. Lipoxin A₄ and Lipoxin B₄ attenuate adipose tissue inflammation in obese patients

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Background: Visceral obesity and adipose inflammation is considered a driving force of systemic disease. Inflammatory resolution is actively regulated by specialized pro-resolving mediators (SPMs), such as the arachidonic acid derived Lipoxin A₄ (LXA₄) and Lipoxin B₄ (LXB₄). We recently demonstrated that LXA₄ attenuates obesity-induced adipose inflammation in mice, resulting in protection against liver and kidney disease (Börgeson *et al*, Cell Metabolism, 2015). The current study attempts to translate these findings from rodent to human pathophysiology. **Method:** White adipose tissue explants were obtained from the greater omentum of obese (BMI 35-50), non-diabetic, bariatric surgery patients (n=4). The adipose tissue was incubated *ex vivo* with vehicle, LXA₄ (1 nM) or LXB₄ (1 nM) for 6 hours at 37°C. Supernatant IL-6 and TNF-α levels were determined using ELISA, and tissue leukocytes were isolated and characterized by flow cytometry. Patients were recruited in accordance with the Helsinki Declaration; ClinicalTrials.gov #NCT02322073. **Results:** In adipose explants from obese patients, lipoxins increased the percentage of CD206⁺ M2 MΦs (LXA₄ +66%, LXB₄ +57%), although CD11c⁺ expression on MΦs was not altered. Importantly, lipoxin treatment reduced TNF-α levels (p<0.05), which is a key functional response in promoting metabolic health. Lymphocyte CD4⁺ and CD8⁺ remained unaltered, although LXA₄ reduced CD69⁺ expression, suggesting a less activated T-cell phenotype. Current experiments further delineate the molecular mechanisms involved in the lipoxin-mediated attenuation of adipose inflammation. **Conclusion:** The data indicate that both LXA₄ and LXB₄ reduce obesity-induced inflammation in human adipose tissue. This encouraging proof of concept study suggests that lipoxins may have therapeutic potential in attenuating metabolic disease in humans. **Acknowledgment:** EB is supported by the Swedish Research Council, Swedish Society for Medical Research (SSMF), Åke Wibergs Foundation, Konrad & Helfrid Johansson Foundation, Wilhelm & Martina Lundgrens foundation, Goljas memorial fund. KS is supported by the NIH and CG by Science Foundation Ireland.

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22. Investigation of the regulation of chronic inflammation in endothelial cells by a pro-inflammatory epoxyisoprostane phospholipid

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Oxidation products of 1-palmitoyl-2-arachidonoyl-*sn*-glycerol-3-phosphatidylcholine (PAPC), referred to as OxPAPC, accumulate in areas of chronic inflammation and regulate over 1000 genes in human aortic endothelial cells, affecting many pathways, including inflammation and monocyte recruitment. It is hypothesized that PEIPC, the most active component of OxPAPC, binds with a mediating protein to activate a biological mechanism that triggers an upregulation of the gene that codes for MCP-1. Potential candidate proteins include GRP-78, VEGFR2, and EP2; however, GRP-78 has proven to be the most likely candidate that binds to PEIPC based off of previous binding studies to Ox-PAPC. This study will investigate the unknown proteins that bind to PEIPC and test regulation of IL-8, HO-1, and MCP-1, representing the inflammatory, oxidative stress, and monocyte recruitment pathways. Binding of PEIPC to endothelial proteins was detected using biotin tagged lipid and Western blotting, and gene regulation in HAECs was tested using real time PCR. We anticipate that PEIPC regulation in endothelial cells is mediated by GRP78, and in the future we plan to compare the mechanism of PEIPC regulation with other chronic inflammatory mediators, including IL1β and TNF-α.

23. Lipidomic phenotyping of severe asthma

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Severe asthmatics form a heterogeneous patient group with progressive airways obstruction, frequent exacerbations, and a high risk of mortality. Our current inability to predict the clinical efficacy of treatments for severe asthma forms an important bottleneck in the development of new drugs. Thus, the aim of the pan-European research project UBIOPRED (*Unbiased biomarkers for prediction of respiratory disease outcomes*) is to unravel the pathophysiological and molecular mechanisms that drive the different disease entities that are collectively known as 'asthma'.

With the active role that lipids play in cellular homeostasis, metabolism and signaling, they present an attractive target for disease biomarker discovery studies. The role of the unbiased lipidomics platform within the UBIOPRED project is thus to find lipid biomarkers that are either predictive of asthma severity, or can be used to stratify the severe asthma cohort. We have used a combination of high-throughput (direct infusion MS) and in-depth (UHPLC-MSⁿ) approaches to characterize the plasma and pulmonary surfactant lipidomes of 620 mild, moderate and severe asthmatics, as well as healthy controls. Combined with the extensive clinical information and the data generated by the other 'omics platforms within UBIOPRED, this has generated lipid 'fingerprints' that are characteristic of different phenotypes of this disease and can be used in future drug development.

24. The precise structures and stereochemistry of trihydroxy-linoleates esterified in human and porcine epidermis and their significance in skin barrier function: Implication of an epoxide hydrolase in the transformations of linoleate

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Creation of an intact skin water barrier, a prerequisite for life on dry land, requires the lipoxygenase-catalyzed oxidation of the essential fatty acid linoleate, which is esterified to the ω -hydroxyl of an epidermal-specific ceramide. Oxidation of the linoleate moiety by lipoxygenases is proposed to facilitate enzymatic cleavage of the ester bond, releasing free ω -hydroxy ceramide for covalent binding to protein, thus forming the corneocyte lipid envelope, a key component of the epidermal barrier. Herein we report the transformations of esterified linoleate proceed beyond the initial steps of oxidation and epoxyalcohol synthesis catalyzed by the consecutive actions of 12R-LOX and eLOX3. The major end-product in human and porcine epidermis is a trihydroxy derivative, formed with a specificity that implicates participation of an epoxide hydrolase in converting epoxyalcohol to triol. Of the sixteen possible triols arising from hydrolysis of 9,10-epoxy-13-hydroxy-octadec-enoates, using LC-MS and chiral analyses we identify and quantify specifically 9R,10S,13R-trihydroxy-11E-octadecenoate as the single major triol esterified in porcine epidermis and the same isomer with lesser amounts of its 9R diastereomer in human epidermis. The 9R,10S,13R-triol is formed by S_N2 hydrolysis of the 9R,10R-epoxy-13R-hydroxy-octadecenoate product of the LOX enzymes, a reaction specificity characteristic of epoxide hydrolase. The high polarity of triol over the primary linoleate products enhances the concept that the oxidations disrupt corneocyte membrane lipids, promoting release of free ω -hydroxy-ceramide for covalent binding to protein and sealing of the waterproof barrier.

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25. ω -Alkynyl arachidonic acid promotes anti-inflammatory M2 polarization of macrophages against acute myocardial infarction via attenuating the expression of iNOS and PKM2

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Delayed resolution of inflammation following acute myocardial infarction exacerbates heart injury and impairs cardiac repair. Macrophages exhibit either pro-inflammatory M1 or anti-inflammatory M2 phenotype, and thereby play key roles in acute myocardial infarction. The aim of the present study was to investigate whether ω -alkynyl arachidonic acid could regulate phenotypic and functional switch of macrophages in myocardial infarction. We initially discovered that ω -alkynyl arachidonic acid selectively suppressed the up-regulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in LPS-stimulated macrophages. We also found that ω -alkynyl arachidonic acid not only reduced the expression of M1 markers (TNF- α , CXCL10, iNOS and IL-6) but also increased the expression of M2 markers (IL-10 and arginase I) in LPS-stimulated macrophages. Moreover, ω -alkynyl arachidonic acid markedly enhanced the phagocytosis of fluorescently-labeled beads or apoptotic H9c2 cardiac cells. These results stimulated us to further investigate the cardioprotective activities of ω -alkynyl arachidonic acid in a mouse model of myocardial infarction. ω -Alkynyl arachidonic acid indeed reduced infarct size, cardiac damage and the release of myocardial enzymes CK-MB. To elucidate the underlying mechanisms, we intended to identify the covalent ω -alkynyl arachidonic acid-protein adducts. By performing biotinylation to the cellular proteins via "click chemistry" alkyne-azido cycloaddition, we isolated glycolytic enzyme pyruvate kinase M2 (PKM2) as a predominant ω -alkynyl arachidonic acid binding protein. ω -Alkynyl arachidonic acid could also attenuate PKM2 expression and suppress nuclear translocation of PKM2 in LPS-stimulated macrophages. Thus, ω -alkynyl arachidonic acid may promote anti-inflammatory M2 polarization of macrophages against acute myocardial infarction via regulating the expression of PKM2 and iNOS.

26. Immunoresponsive gene 1 and itaconate modulate inflammation via succinate dehydrogenase inhibition

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Metabolic reprogramming is emerging as a hallmark of the innate immune response, and the dynamic control of metabolites such as succinate serves to facilitate the execution of inflammatory responses in macrophages and other immune cells. Immunoresponsive gene 1 (*Irg1*) expression is induced by inflammatory stimuli, and its enzyme product *cis*-aconitate decarboxylase (CAD) catalyzes the production of itaconate from the tricarboxylic acid (TCA) cycle. Here we identify an immunometabolic regulatory pathway that links *Irg1* and itaconate production to the inflammatory response in macrophages. Itaconate levels and *Irg1* expression correlate strongly with succinate during lipopolysaccharide (LPS) exposure in macrophages and non-immune cells. We demonstrate that itaconate acts as an endogenous succinate dehydrogenase (SDH) inhibitor to cause succinate accumulation. Modulation of itaconate levels or *Irg1* expression in activated macrophages influences the expression of immune related genes such as *Il-1 β* . This metabolic network links the innate immune response, TCA metabolism, and inflammatory disease pathogenesis.

27. Obesity is associated with impaired B cell activation and antibody production in humans upon TLR9 plus BCR stimulation

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Obesity promotes a diminished response to vaccinations and infections. Therefore, understanding how obesity targets B cell-driven humoral immunity, particularly at a mechanistic level, is essential to elucidate. In rodent studies, our data has revealed that obesity impairs the humoral immune response. Herein, our work has expanded to show that obese males with a BMI of >30 have an elevated percentage of B cells in circulation with no change in the percentage of monocytes, helper T cells, and cytotoxic T cells. Co-stimulation of B cells with TLR9 and B cell receptor (BCR) agonists led to a decrease in IL-6 secretion, compared to B cells of lean individuals. In addition, a positive correlation was observed between BMI and IgM but not IgG production upon B cell stimulation with anti-TLR9/anti-BCR stimulation. To explore underlying mechanisms, lipidomic analyses were conducted. The resulting lipidomic profile of the obese individuals, similar to mice, revealed generally elevated levels of arachidonic acid-derived lipid mediators, potentially contributing to a chronic inflammatory state. Overall, our results suggest that defective B-cell responses likely contribute toward an obese individual's compromised response to vaccination and infection, potentially driven by changes in the arachidonic acid lipidome.

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28. WITHDRAWN

29. Gamma-linolenic acid therapy of glioma

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Glioblastoma multiforme (GBM), a common primary malignant brain tumor, has a median survival of ~ 15 months, with only 3%–5% surviving longer than 36 months. GBM is resistant to treatment despite a debulking surgery, radiation and chemotherapy. Tumor cells have low rates of lipid peroxidation compared with normal cells, whereas lipid peroxidation decreases with increasing growth rate. This low rate of lipid peroxidation is attributed to their low polyunsaturated fatty acids (PUFAs) content as a result of decreased activity of Δ^6 and Δ^5 desaturases and elevated levels of antioxidant α -tocopherol. Previously, I showed that γ -linolenic acid (GLA) selectively kills tumor cells by enhancing free radical generation and lipid peroxides. GLA induces apoptosis of cancer cells with no toxic action on normal cells. GLA is effective against a rat C6 glioma implantation model with no histological tissue damage to normal cells. GLA decreased the survival of 36B10 malignant astrocytoma cells compared to control and enhanced cytotoxic action of gamma- radiation on malignant astrocytoma cells but not of 'normal' astrocytes. GLA up-regulated miRNA target genes associated with apoptosis in glioma cells. C6 rat gliomas treated for 14 days with GLA decreased tumour size associated with reduced expression of vascular endothelial growth factor (VEGF) and VEGF receptor Flt1 but not Flk1, and expression of ERK1/2 was also reduced. GLA altered expression of several proteins involved in cell cycle control. pRb protein expression was decreased. The expression of p53 was increased by GLA. BrdU incorporation was inhibited into the tumour *in vivo*. Overall, GLA inhibited glioma cell proliferation *in vivo* and showed a direct effect upon cell cycle control and angiogenesis.

30. Arachidonic acid may function as an endogenous anti-diabetic molecule

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Identifying factors that preserve pancreatic β cells may form a new approach to prevent and manage both type 1 and type 2 diabetes mellitus. Studies showed that ω -3 eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) and their anti-inflammatory metabolites resolvin E1 and protectin D1 respectively possess insulin-sensitizing and antisteatotic actions in the *ob/ob* mice. It was reported that high pancreatic n-3 fatty acids prevent streptozotocin-induced diabetes in *fat-1* mice. In the present study, we noted that both ω -6 and ω -3 PUFAs can prevent both alloxan and streptozotocin (STZ)-induced apoptosis of rat-insulinoma (RIN) cells *in vitro*. Of all the PUFAs tested, AA was found to prevent both alloxan and STZ-induced apoptosis and alloxan-induced diabetes *in vivo* that was not blocked by both cyclo-oxygenase and lipoxygenase inhibitors suggesting that prostaglandins, leukotrienes and thromboxanes do not have a role in this process. This was confirmed by the observation that various prostaglandins, leukotrienes and thromboxanes did not prevent both alloxan and STZ-induced apoptosis of RIN cells *in vitro*. Lipoxin A4 (LXA4), an anti-inflammatory product of AA, prevented alloxan and STZ-induced apoptosis of RIN cells *in vitro* and alloxan and STZ-induced diabetes mellitus in experimental animals. AA and other PUFAs enhanced formation of LXA4 in RIN cells *in vitro*. Plasma phospholipid content of AA was decreased in alloxan-treated animals and patients with type 2 DM. Preliminary studies showed that plasma LXA4 are low in patients with type 2 DM. These results suggest that AA and its anti-inflammatory product LXA4 may function as endogenous anti-diabetic molecules.

31. Pancreatic cancer cells scavenge lysophospholipids from stroma in the hypoxic tumor microenvironment

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Pancreatic ductal adenocarcinoma (PDAC) is non-resectable in 85% of cases and highly resistant to chemotherapy, resulting in a poor 5-year survival (5-7%). Understanding the metabolic vulnerabilities of PDAC in the harsh tumor microenvironment may lead to novel therapeutic approaches with improved clinical efficacy. Up to 90% of the pancreatic tumor mass consists of non-neoplastic cells, and high interstitial pressures and poor perfusion both result in severe hypoxia, leading to a more malignant PDAC phenotype. We hypothesized that these conditions lead to specific metabolic constraints in oncogene-driven, rapidly proliferating PDAC cells that experience high levels of nutrient stress, in contrast to the surrounding quiescent stromal cells. We used co-culturing of PDAC (MIAPaCa2) and stromal (NIH/3T3) cells in transwell systems as a robust and reproducible model of cell contact-independent interactions in the tumor microenvironment. A commercial metabolic profiling platform (Metabolon) and ¹³C-based flux assays were used to study changes in metabolite levels in both cell types in normoxia or hypoxia (1% O₂). We found that hypoxia induced similar metabolic changes in the PDAC and stromal cells. Interestingly, the metabolic effects of co-culturing were predominantly observed in the stromal compartment, e.g. enhanced glycogenolysis, metabolite changes indicative of gluconeogenesis, and increased dipeptide levels, all reminiscent of a 'starvation' phenotype. In contrast, the tumor cells maintained a mixed anabolic and catabolic phenotype, as shown by elevated intracellular levels of essential amino acids and ribonucleotide triphosphates, representative of a 'feeding' phenotype. Importantly, a unique dependence on lysophospholipids was observed in cancer cells with reciprocal changes in stromal cells. These data were confirmed in portal vein plasma samples isolated from pancreatic cancer patients before and after surgery. These data suggest that pancreatic cancer cells reprogram stromal cells to 'feed off' the metabolic capacity of non-neoplastic cells, in particular diffusible lysophospholipids, to satisfy their specific catabolic needs.

32. Biochemical characterization and inhibitor discovery of *Pseudomonas aeruginosa* 15-lipoxygenase enzyme

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Lipoxygenases (LOXs) are a family of non-heme iron-containing enzymes found abundantly in both the plant and animal kingdoms, as well as in some bacteria. In humans LOXs catalyze the dioxygenation of polyunsaturated fatty acids, which is the first step in a variety of biosynthetic pathways with implications in immune disorders, inflammation, and cancers. *Pseudomonas aeruginosa* (*P. aeruginosa*), one of the main bacteria responsible for death in cystic fibrosis patients, has recently been discovered to produce and secrete a functional 15-lipoxygenase (15-LoxA), whose transcription is up-regulated more than 200-fold during biofilm formation. We have shown that the secreted lipoxygenase and its enzymatic activity may be necessary for biofilm formation and viability of *P. aeruginosa* in human airway cells. Regulation of 15-LoxA activity, via small molecule inhibitors, may potentially play a key role in the prevention of patient death due to *P. aeruginosa* infections.

33. The role of lysophosphatidic acid on hepatic insulin signaling

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Hepatic insulin resistance is a major cause in the pathogenesis of type 2 diabetes mellitus (T2DM). It is characterized by hyperinsulinemia, hyperglycemia and hypertriglyceridemia. Obesity which is associated with adipocyte hypertrophy plays a crucial role in the development of hepatic insulin resistance and T2DM. Little is known concerning the molecular mechanisms responsible for the interaction between adipose tissue and the liver. Adipose tissue regulates the insulin-sensitivity and energy homeostasis of peripheral tissues via the secretion of adipokines such as the exoenzyme autotaxin (ATX) which is enhanced in obesity. ATX converts lysophosphatidylcholine into the bioactive lipid mediator lysophosphatidic acid (LPA) which induces intracellular signaling after binding specific Receptors (LPA₁₋₆).

To examine the role of LPA on hepatic insulin signaling, primary rat hepatocytes were used. The interaction between LPA and insulin signaling was analyzed via insulin-mediated glucokinase-expression, PI₃K-activation and glycogen synthesis as markers for insulin sensitivity. In these cells insulin-induced the activation of PI₃K and it was accompanied by an enhanced expression of glucokinase and glycogen synthesis. Importantly, pretreatment of hepatocytes with LPA (18:1 and 16:0) resulted in an inhibition of insulin-mediated glucokinase-expression, PI₃K-activation and glycogen synthesis. Real-time PCR revealed that all LPA-receptor subtypes are present in primary rat hepatocytes except LPA₄ and LPA₅. Pharmacological approaches reveal that the LPA₃-receptor subtype is responsible for the inhibitory effect of LPA on insulin signaling via inhibition of glucokinase-expression and glycogen synthesis.

Taken together LPA was able to interrupt insulin-induced glucokinase-expression, PI₃K-activation and glycogen synthesis in hepatocytes. Pharmacological intervention to interrupt LPA-receptors revealed the crucial role of LPA₃-receptor in insulin sensitivity. Thus, LPA₃ inhibition could be considered as a novel therapeutic target for the treatment of insulin resistance.

34. Development of an improved protocol for lipid analysis based on heat inactivation of enzymes.

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The removal of a biological sample induces a cascade of reactive changes causing alterations to the molecular profile of the sample. This is particularly true for metabolomic studies where sample preservation is key for the relevance of the analytical results. In order to avoid this and enable analysis of a molecular state closer to *in vivo* state, heat induced protein denaturation has been introduced as a means to preserve sample composition and quality¹. A study has been done to evaluate the inclusion of heat stabilization in the sample preparation prior to lipid analysis. The study was performed on mouse livers from 30 healthy mice, randomized in 5 groups of 6 animals designed to compare snap freezing at -80°C to 3 heat stabilization conditions (freezing just after heat stabilization, after 1 hour and after 3 hours) and to a negative control samples maintained at room temperature during 10 minutes before freezing. For each mouse liver apolar lipids were extracted and analyzed using LC-MS, on a Q-Exactive Orbitrap and a Maxis HD Q-Tof, for integrated lipid profiling. Representative lipids profiles were generated and compared between the different treatment groups. Analysis demonstrates that heat stabilization is equivalent and for a range of lipids even better than traditional freezing for lipidomics. This has been verified using relative quantification on phospholipids, fatty acids and other relevant lipids. A new biological sample preservation procedure based on heat stabilization for improved lipid profiling has been developed.

Reference

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35. Acyl acyl carrier protein synthetases – A new tool to study lipids

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The acyl carrier protein (ACP) is central to numerous metabolic pathways, including fatty acid, polyketide and nonribosomal peptide biosynthesis. ACP requires posttranslational modification with a 4'-phosphopantetheine arm for activity, and this thiol-terminated modification carries cargo between enzymes in these pathways. Acyl-ACP synthetases (AasSs) are adenylate-forming enzymes that load fatty acids on to the posttranslationally modified ACP (holo-ACP), and we postulated that this class of enzymes might have some inherent flexibility. We showed that the *Vibrio harveyi* AasS is able to load even, odd and unnatural fatty acids onto *E. coli* ACP in vitro. *Vibrio harveyi* AasS not only shows promiscuity for the acid substrate, but also the ACP substrate, active upon various alternate carrier proteins from FAS and PKS pathways. Curious if the AasS technology extended in vivo, fatty acids with terminal bromine, phenyl and azide moieties were fed to *E. coli* overexpressing the *Vibrio harveyi* AasS. These exogenously supplied unnatural fatty acid analogs were loaded onto ACP, extended by the *E. coli* fatty acid synthase, and integrated into cellular lipids. We also studied this technology in other organisms, including green microalgae *C. reinhardtii* and cyanobacteria *Synechocystis*. Facile access to unnatural modified ACPs in vivo via AasS may hold the key to unique structural studies in various organisms.

36. Cardioprotection following injury heart failure afforded by a non-enzymatic oxygenated metabolite of omega 3 fatty acid involves Ryanodine receptor mechanism and mitochondrial function.

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Cardioprotective effects of long-chain polyunsaturated fatty acids of the n-3 series (PUFAs) have been demonstrated and represent a novel approach to prevent myocardial infarctions or its consequences. Due to the abundance of double bonds, the main n-3 PUFAs; docosahexaenoic acid (C22: 6 n-3, DHA) are very sensitive to free radical oxidation and can undergo non-enzymatic spontaneous peroxidation under oxidative stress conditions as it occurs in ischemia/reperfusion. In this context, a lot of oxygenated metabolites of PUFAs like Neuroprostanes (NeuroPs) are produced and used as oxidative stress biomarkers but their activities were not determined. We investigated if the pericardial delivery of NeuroPs, protects the myocardium from ischemic damages during and following an ischemia/reperfusion (IR) episode in rats. Cardiac functions, infarct size and arrhythmias were studied and we observed that NeuroPs afford some cardioprotective effect during or after myocardial infarction. Indeed, compared with controls, NeuroPs-treated animals have significantly decreased infarct size (-28%) determined at the end of reperfusion and reduced ventricular arrhythmia score during reperfusion (-38%). Mechanistically, NeuroPs regulates calcium levels by stabilizing RyR2 activity (Roy et al., 2015), which can explain arrhythmias prevention during IR. Also, our results demonstrated an increase of membrane potential ($\Delta\Psi_m$) by the application of NeuroPs. This effect was not due to an augmentation of mitochondrial respiratory chain activity but by the effect leading to the diminution of protons leak. Swelling in response to Ca^{2+} was prevented by NeuroP, indicating a decrease MPTP opening, which can be explain prevention of cell death during IR. These results suggest a novel pharmacological pathway of n-3 PUFAs and suggest that their well-known cardioprotective effects are mediated by their oxygenated metabolites such as NeuroPs.

37. High performance liquid chromatography coupled with tandem mass spectrometry to investigate eicosanoids profile in peripheral blood after stimulation: a comparison between sickle cell anemia patients in treatment and healthy individuals

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The metabolites from arachidonic acid, such as prostaglandins (PGs) and leukotrienes (LTs), play an important role in homeostasis and in pathogenesis of various human diseases and several pharmacological agents can stimulate their biosynthesis. *Objective:* was to standardize a method to determine the eicosanoid profile in human plasma samples after whole blood stimulation, accurate enough to assessing differences between healthy and sick individuals. *Methods:* a liquid chromatography-tandem mass spectrometry method was validated for the quantification of 22 eicosanoids using human plasma, and the method for the stimulation of whole human blood was optimized. *Results:* In mass spectrometry method, the linearity assays presented regression coefficient ≥ 0.98 for all eicosanoids. The mean intra-assay and inter-assay accuracy and precision values had relative standard deviations and relative errors of $\leq 15\%$, except for the lower limit of quantification ($\leq 20\%$). For blood stimulation, A23187 and thapsigargin were the most potent stimuli to induce eicosanoids generation. When compared the eicosanoid profiles of healthy volunteers with that from patients with sickle cell anemia, under treatment with hydroxyurea or after red blood cell transfusion, we found that healthy subjects produce less 5-HETE, 12-HETE, LTB₄ and TXB₂, and more LTE₄, and PGE₂. *Conclusion:* our analytical method is suitable for identify and quantify changes in eicosanoid profiles released in whole blood after *in vitro* stimulation. These methods may contribute for investigate eicosanoid alterations in several inflammatory and infectious diseases.

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38. Comparison of the anti-inflammatory properties of selected DHA-derived oxylipins and insights into their mechanism of action.

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The anti-inflammatory properties of DHA have been largely demonstrated *in vitro* and *in vivo* but research gaps remain regarding the contribution of its oxygenated metabolites also called oxylipins. We aimed to investigate the anti-inflammatory properties and potential mechanisms of action of different types of DHA-derived oxylipins including Neuroprostanes (NeuroP), Protectin DX (PDX) as well as Neuroprotectin D1 (NPD1/PD1) and its isomer 10S,17S-diH n-3 DPA_{EEZ}. Human peripheral blood mononuclear cells were isolated from healthy donors by Ficoll density gradient centrifugation. Monocytes were differentiated into resting macrophages (RM) for 7 days. RM were exposed to the different types of oxylipins (i.e. 14-A₄-NeuroP, 4(RS)-4-F_{4t}-NeuroP, PDX, PD1 and 10S,17S-diH n-3 DPA_{EEZ}) at 3 different doses (i.e. 0.1, 1 and 10 μ M) during 30 min. The inflammatory response was then induced with LPS (100 ng/mL) for 6 hours. Preliminary results of gene expression analysis (qPCR) show that IL-6, MCP-1, COX-2, TNF α or CCL3 mRNA were significantly lower in macrophages pre-exposed to 10 μ M 14-A₄-NeuroP (-84%, -57%, -29%, -41% and -23% respectively). Significant but less pronounced effects on IL-6 and MCP-1 were also observed with 10 μ M 4(RS)-4-F_{4t}-NeuroP (-25% and -25% respectively). Reduced levels of TNF α protein secretion (ELISA) were found in macrophages pre-exposed to 10 μ M 4(RS)-4-F_{4t}-NeuroP (-12% p<0.05) while measurable but less pronounced effects were observed with 14-A₄-NeuroP, PDX, PD1 or 10S,17S-diH n-3 DPA_{EEZ} (-9%, -22%, -10% and -15% ns, respectively). Abundance and phosphorylation of I κ B α (Western Blot) suggest that 14-A₄- and 4(RS)-4-F_{4t}-NeuroPs could exert their anti-inflammatory effects through the inhibition of I κ B α phosphorylation. Finally, cotransfection of luciferase reporter vector with human PPAR γ expression vector performed in Cos-7 cells suggests that 14-A₄- and 4(RS)-4-F_{4t}-NeuroPs probably act independently of PPAR γ . In conclusion, these results suggest that the anti-inflammatory properties of DHA could be mediated, at least in part, by oxylipins, and bring new insights into their mechanism of action.

39. Oxylipidomics of human platelets: Navigating the challenges of oxylipid mass spectrometry

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The oxylipidome is a massive network of inflammatory lipid mediators (LMs). This network is of immense interest in that its regulation is still being deciphered and is implicated in the pathology of many diseases, such as cardiovascular disease and cancer. Thus, a simple method to determine the flux of LMs in a given biological system would be extremely helpful in teasing out regulatory hubs. Although the literature is rife with LC-MS/MS methods for the targeted measurement of LMs, we have encountered several complications to the implementation of these methods: particularly, a lack of translatable identification data. These LMs are a challenge to identify and measure, given that many species are isobaric, share many MS2 fragments, are difficult to separate chromatographically, and are of too low concentration in biological samples to collect UV-Vis data. Additionally, the current dearth of predictive identification software restricts oxylipid measurements/identification to available standards. Using oxylipid standards, we have developed an UPLC-LIT/Orbitrap method for the targeted measure of 36 oxylipids. This method spans the major arachidonic acid metabolome and is sensitive to 10pg injected LM. We have measured this targeted oxylipidome in platelet samples and hope to probe the flux of oxylipids in this *ex vivo* system and other systems in the future.

40. Strict regio-specificity of human epithelial 15-lipoxygenase-2 delineates its transcellular synthesis potential

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Lipoxins are an important class of lipid mediators that induce the resolution of inflammation, and arise from transcellular exchange of arachidonic acid (AA)-derived lipoxygenase products. Human epithelial 15-lipoxygenase-2 (h15-LOX-2) –the major lipoxygenase in macrophages– has exhibited strict regio-specificity catalyzing only the hydroperoxidation of AA's carbon 15. To determine the catalytic potential of h15-LOX-2 in transcellular syntheses events, we reacted it with the three lipoxygenase-derived monohydroperoxy-eicosatetraenoic acids (HPETE) in humans: 5-HPETE, 12-HPETE, and 15-HPETE. Only 5-HPETE was a substrate for h15-LOX-2, and the steady-state catalytic efficiency (kcat/Km) of this reaction was 31% of the kcat/Km of AA, which is significantly greater than the typical catalytic efficiencies of HPETEs with other lipoxygenases. The only major product of h15-LOX-2's reaction with 5-HPETE was the proposed lipoxin intermediate, 5,15-dihydroperoxy-eicosatetraenoic acid (diHPETE). We performed DFT calculations to determine the stability of the radical resulting from the abstraction of the C10 hydrogen atom from 5,15-diHPETE and found that it was 5.4 kJ/mol more stable than the radical from AA, demonstrating the facility of this intermediate for lipoxin formation. Even so, h15-LOX-2 could not produce lipoxins from 5,15-diHPETE. When reacted with LTA4 or 5(S),6(R)-diHPETE however, h15-LOX-2 synthesized lipoxin A4. Taken together, these results demonstrate the strict regiospecificity of h15-LOX-2 that circumscribes its role in transcellular synthesis.

41. Docosahexaenoic acid improves the decrement in antibody production associated with murine obesity upon influenza infection through the production of CD138⁺ cells

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Obesity is associated with increased risk for infections and poor responses to vaccination, which may be due to diminished B-cell responses. Based on our previous observations of the benefits of n-3 polyunsaturated fatty acids (PUFA) on B-cell antibody production, we hypothesized that the n-3 PUFA docosahexaenoic acid (DHA) could improve the humoral immune response to influenza infection in a mouse model of obesity. Our findings reveal that DHA supplementation in a mouse diet improved hemagglutination inhibition (HAI) titers accompanied by an improved body weight loss after influenza A/PR/8/34 infection. DHA had no effect on HAI titers in the lungs although lung viral transcripts were significantly lowered compared to the control. Mechanistically, the specialized pro-resolving lipid mediator 17-HDHA, synthesized from DHA, is reported to boost antibody production by increasing the frequency of CD138⁺ plasma cells. Flow cytometry analyses revealed DHA increased the frequency of CD138⁺ cells in the bone marrow compared to the high fat diet. Lipidomic analyses showed a significant increase in the levels of several SPMs that can boost antibody levels. Overall, our work suggests that dietary supplementation with DHA could be a valuable therapeutic strategy to improve the immune response of the obese against influenza infection.

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42. Resolvin D1 modulates macrophage plasticity to promote resolution of inflammation

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Our recent report suggests that specialized bioactive lipid-mediator Resolvin D1 (RvD1) expedites resolving phase after myocardial infarction (MI) by coordinating neutrophils and macrophages clearance. However, it is unclear whether RvD1 has potential to regulate macrophage phenotype and plasticity. Here, we tested and compared the potency of RvD1 bioactive to parent molecule DHA (Docosahexaenoic acid) and arachidonic acid-derived pro-inflammatory-metabolite 12-(S)-HETE (12-hydroxyeicosatetraenoic acid) on macrophage phenotypes. Peritoneal macrophages were isolated from male C57BL/6 (2-4 months) mice and treated with RvD1 (10ng/ml), DHA (50 μM) and 12-(s)-HETE (100 nM) for 4, 8, 12 and 24 hrs. RvD1 down regulated, mRNA expression of proinflammatory markers *TNF-α*, *IL-6*, *CCL2* and *IL-β* at 4 hrs compared to DHA and 12-(S)-HETE. *TNF-α*, *IL-6* and *IL-1β* expression continued to be elevated in macrophages treated with DHA and 12-(S)-HETE up to 24 hrs. RvD1 treated peritoneal macrophages displayed increase in expression of proresolving phenotype *ARG1* (12 fold; $p<0.05$), *YM-1* (2 fold; $p<0.05$) and *MRC-1* (9 fold $p<0.05$) compared with DHA and 12-(S)-HETE. RvD1 activated 5-lipoxygenase protein expression compared with DHA and 12-(S)-HETE. In summary, RvD1 bioactive is more potent than parent DHA and 12-(S)-HETE chemokines that modulate macrophage plasticity and response, thereby regulating cellular microenvironment.

43. HILIC-IM-MS of eye tissue from a mouse Bietti's model reveals decreases in phospholipids

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Bietti's crystalline dystrophy (BCD) is a degenerative eye disease characterized by the presence of crystalline lipid deposits in the retina and cornea. These deposits result in a progressive decline in central vision, visual field loss, and the development of night blindness. While the genetic mutations responsible for BCD have been localized to the *CYP4V2* gene, little is known about the composition of the yellow-white crystalline lipid deposits that define the morphology of BCD. An untargeted lipidomics strategy comprising of hydrophilic interaction chromatography (HILIC) and ion mobility-mass spectrometry (IM-MS) with data-independent tandem MS (MS/MS) was used to characterize the lipid compositions of wild-type (WT) and *Cyp4v3*^{-/-} (KO) mouse eye. The HILIC-IM-MS/MS approach provided rapid chromatographic and gas-phase separation of lipids by polarity and structure, respectively, and resulted in enhanced signal-to-noise and higher-confidence assignment of identifications than conventional methods. Collision cross sections obtained from the IM separation added orthogonal information to validate the identification. The differences between lipid profiles of KO and WT eyes was visually-evident as the KO eyes displayed a significant decrease in the chromatographic peaks associated with phospholipids such as phosphatidylcholines (PCs), phosphatidylserines, phosphatidic acid and lysophosphatidylethanolamines. Bioinformatics analysis confirmed the decreased abundance of the polar phospholipid species in KO eyes, and revealed a number of small nonpolar lipid species that were increased in the KO eyes. Some of the major differentially expressed polar lipid species were identified as PCs containing docosahexaenoic acid (DHA, C22:6), stearic acid (C18:0), oleic acid (C18:1) and palmitic acid (C16:0), which are the most prevalent fatty acids in the retina. These findings indicate a disruption of lipid homeostasis in the eyes of a mouse model for BCD, which results in a biological environment that is enriched with more nonpolar lipids.

44. Prostaglandin E-2 promotes human colorectal cancer cell survival against oxaliplatin

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Oxaliplatin, a platinum-based DNA crosslinking agent, has been used successfully as a first-line chemotherapeutic agent for treatment of metastatic colorectal cancer (CRC). Long-term treatment with oxaliplatin results in resistance in many patients, limiting its therapeutic efficacy. Previous studies have shown that inhibition of cyclooxygenase-2 (COX-2) enhances chemotherapeutic drug efficacy in CRC. The major COX-2 product, prostaglandin E-2 (PGE2), has been implicated in colorectal tumorigenesis; however, it is unknown whether PGE2 plays a direct role in chemoresistance. Here we investigated the relationship between PGE2 and oxaliplatin sensitivity using human CRC cell lines, HT29 and HCT116. Oxaliplatin-resistant HT29 cells (HT29-OXR) were generated by chronic exposure of parental HT29 cells to increasing concentrations of oxaliplatin. Compared to parental cells, HT29-OXR cells produce significantly higher (3-fold) levels of PGE2, associated with elevated expression of COX-2 (18-fold) and the terminal PGE2 synthase, microsomal prostaglandin E synthase-1 (mPGES-1) (7-fold). siRNA silencing of mPGES-1 in HT29-OXR cells increased oxaliplatin sensitivity (IC50) by 30%, measured by MTT cell viability. We next tested conditioned media (CM) from HT29-OXR cells and parental cells and found that oxaliplatin-resistant CM increased survival of HT29 parental cells. This paracrine effect was abrogated by blockade of EP2 or EP4 receptor signaling using selective pharmacologic antagonists. Moreover, we have demonstrated that the addition of exogenous PGE2 to both HT29 and HCT116 cells significantly decreases their oxaliplatin sensitivity. FACS analysis showed that co-treatment of HT29 cells with exogenous PGE2 for 24 hours leads to an ~25% reduction in oxaliplatin-induced Annexin V staining for early apoptosis; in HCT116 cells, exogenous PGE2 maintained a G2/M arrest, concurrently reducing proportion of dead sub-G1 cells by ~80%. Overall, our findings uncover an important role for PGE2 signaling in human CRC cell survival following oxaliplatin treatment, possibly *via* regulation of apoptosis, and provide new data suggesting that targeting mPGES-1 may be a useful strategy for increasing oxaliplatin efficacy.

45. Immunoassays and mass spectrometry: A transition

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Immunoassays, such as enzyme-linked immunosorbent assays (ELISA), rely on the specificity of the antibody-antigen interaction for the detection and quantification of biomolecules of interest. It has recently come into the spotlight that poorly characterized antibodies have given rise to erroneous results in the research community (and in some cases, clinical labs). It is also known that structurally related molecules or interferents can alter binding of the analyte of interest with well characterized antibodies causing misleadingly high or low (incorrect) results. This has created a challenge for researchers quantifying small molecules and biomolecules such as eicosanoids in biological samples. Researchers and clinicians alike are now turning to mass spectrometry (MS) as an alternative analytical technique because of its potential advantages over ELISA-based methods. MS offers an inherent specificity advantage as well as multiplexing capabilities, albeit with significant start-up costs. The value of rapidly quantifying multiple targets simultaneously with accuracy and certainty makes MS an increasingly popular method of choice. Here we present data obtained when measuring eicosanoids by immunoassay and mass spectrometry (LC-MS/MS). It briefly considers the limitations of each technology and describes the potential benefits of combining these two technologies, i.e. using antibodies to improve the sensitivity of mass spectrometry-based assays.

46. Lipidomic analyses identify mitochondrial lipids and omega-3/omega-6 phospholipid decreases in a mouse model of Gulf War Illness

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Introduction: Gulf war illness (GWI) is multi-symptom illness that affects the veterans from the 1991 Gulf War (GW). Using chemicals implicated in the pathogenesis of GWI (a nerve gas antidote pyridostigmine bromide and a pesticide permethrin), we developed a mouse model of GWI which presents with cognitive impairment and anxiety, features that are similar to symptoms reported by veterans with GWI. Since studies suggest that chronic clinical presentation of GWI accompanies impaired energy utilization in the brain and altered inflammatory parameters in affected GWI veterans, we examined brain lipid profiles in this mouse model of GWI at a chronic 16-months post-exposure, a timepoint that is relevant to the current clinical condition of veterans with GWI.

Methods: Brain cardiolipin (CL) were extracted using a modified Bligh-Dyer method and analyzed using normal phase high pressure liquid chromatography (HPLC) followed by mass spectrometry (MS) in the fourier transform mass spectrometry mode at 100,000 resolution on a LTQ-Orbitrap mass spectrometer. Following acetonitrile-methanol crash, acylcarnitines were analyzed using hydrophilic interaction liquid chromatography (HILIC) and MS analysis in the FTMS mode at 30,000 resolution on the LTQ-Orbitrap. Lipid extracts obtained using the Folch method were subjected to normal phase LC/MS to separate phospholipid (PL) classes and individual species were identified and quantified with the LipidomeDB software. Lipid extracts were saponified for gas chromatography/mass spectrometry analysis to examine the total fatty acid (FA) content. **Results:** We observed decreases in acylcarnitine, CL and in PL that contained omega-3 and omega-6 FA in the brains of exposed animals. Total FA analysis also confirmed decreases in omega-3 and omega-6 FA in the brains of GW agent exposed mice. **Conclusion:** These results indicate that further examination of mitochondria and biological functions involved in transport and metabolism of omega-3/omega-6 fatty acids may lead to the development of novel therapies for treating GWI.

47. Non-HDL cholesterol – A surrogate marker for LDL cholesterol in dyslipidemic patients?

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Background: Dyslipidemia implies elevated LDL Cholesterol, Triglycerides and decreased levels of HDL. It is well known and it has been proved that increased LDL has played an outstanding role in assessment of risk of Cardiovascular Disease but some studies have also reported the use of non-HDL Cholesterol in it. Although this parameter has a lot of advantages, it is rarely used by general practitioners in lipid profile assessment. In this study we aimed to compare non HDL Cholesterol with LDL and total Cholesterol as a predictor of cardiovascular risk in patients with Dyslipidemia. **Methods:** Retrospective analysis of lipid profile in 1000 subjects having cholesterol ≥ 200 mg/dl was done. As per the revised National Control Education Plan (NCEP)-Adult Treatment Panel (ATP) III Guidelines, subjects were divided into 5 groups according to their LDL Cholesterol levels. Total Cholesterol, HDL Cholesterol and LDL Cholesterol were measured with enzymatic method on Hitachi Modular P800 whereas Non HDL Cholesterol (Total Cholesterol-HDL Cholesterol), LDL/HDL and TC/HDL ratios were calculated. **Results:** Highly significant levels of non HDL were seen in all the 5 groups even in which LDL Cholesterol levels were normal or slightly raised. Non HDL Cholesterol significantly correlated with total Cholesterol (p value <0.00001), LDL Cholesterol (p value <0.05), TC/HDL ratio (p value <0.00001) and LDL/HDL ratio (p value <0.05) in all the groups. **Conclusion:** Based on the findings of the present study it was observed that the measurement of Non HDL, TC/HDL ratio and LDL/HDL ratio can provide more relevant information to the Cardiovascular risk than LDL alone in Dyslipidemia, So we can simply perform the test for Non HDL Cholesterol which does not even require fasting blood sample rather than waiting for a fasting blood sample to measure LDL Cholesterol.

Keywords: Dyslipidemia, LDL Cholesterol, Non-HDL Cholesterol, TC/HDL-C ratio, LDL-C/HDL-C ratio.

48. Development of analytical methods for intracellular phospholipase A

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Fatty acids of phospholipids are actively remodeled. Namely, phospholipase A₁ (PLA₁) or phospholipase A₂ (PLA₂) hydrolyzes an acyl group from the *sn*-1 position or *sn*-2 position of phospholipids, generating lysophospholipids (LPLs) that is, 2-acyl-1-LPLs or 1-acyl-2-LPLs, respectively. LPLs thus generated are quickly acylated by lysophospholipid acyltransferases (LPLATs) in acyl-CoA-dependent manners. This acyl group remodeling pathway is known as the Lands cycle. Several kinds of PLAs and LPLATs are believed to be involved in this pathway. To know the possible role of PLAs in fatty acid remodeling reaction, it is important to know their cellular substrates. Recently, massspectrometric methods in combination with liquid chromatography (LC-MS/MS) have been developed, which enabled us to examine the substrates of PLAs. In this study, we tried to detect such PLA activity at cellular level using LC-MS/MS. We focused on intracellular PLA₁ (iPLA₁, iPLA₁ α , β , γ), which have been implicated remodeling of phosphatidylinositol. We overexpressed iPLA₁ α or γ in HEK293 cells and determined the LPLs levels by LC-MS/MS. Unexpectedly, no increase in the level of 2-acyl-1-LPLs was observed in iPLA₁-overexpressing cells. However, in the presence of Acyl-CoA synthetase (ACS) inhibitor, interestingly, iPLA₁-dependent increase in the level of 2-acyl-LPLs was detected. In iPLA₁ γ -overexpressing cells, all kinds of LPLs increased. By contrast, 2-acyl-lysophosphatidylethanolamine was selectively upregulated in iPLA₁ γ -overexpressing cells. The present study indicates that detection of cellular PLA activities are only possible when LPLAT reaction is suppressed.

49. S1P in HDL promotes the interaction between SR-BI and S1PR1 and activates S1PR1-mediated biological functions

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HDL (High Density Lipoprotein) normally transports about 50-70% of plasma S1P (Sphingosine 1-Phosphate) and the S1P in HDL reportedly mediates several HDL-associated biological effects and signaling pathways. The HDL receptor, SR-BI, as well as the cell surface receptors for S1P (S1PRs) may be involved partially and/or completely in these HDL-induced processes. It has been suggested that the interaction between HDL and SR-BI may provide the necessary spatial proximity to bring S1P in HDL to its cognate receptors to initiate S1PR-mediated signaling cascades. Here we investigate the nature of the HDL-stimulated interaction between the HDL receptor, SR-BI, and S1PR1 using a protein-fragment complementation assay (PCA) and confocal microscopy. In both primary rat aortic vascular smooth muscle (RVSMC) and HEK293 cells, the S1P content in HDL particles increased intracellular calcium concentration that was mediated by S1PR1. Mechanistic studies performed in HEK293 cells showed that incubation of cells with HDL led to an increase in the physical interaction between the SR-BI and S1PR1 receptors that mainly occurred on the plasma membrane. In HEK293 cells, incubation with recombinant HDL (rHDL) with S1P incorporated into the particles, but not with rHDL alone, initiated the internalization of S1PR1, suggesting that S1P transported in HDL particles can activate S1PR1. In sum, these data suggest that S1P in HDL stimulates the transient interaction between SR-BI and S1PRs that can activate S1PRs and induce an elevation in intracellular calcium concentration.

50. Role of TNF signaling in *de novo* lipogenesis in the liver upon hypernutrition

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Obesity-associated tissue inflammation is thought to be an important cause of decreased insulin sensitivity and glucose intolerance that are the key pathophysiological characteristics of cardiovascular diseases, type 2 diabetes, and non-alcoholic steatohepatitis (NASH). In this study, we will investigate the hypothesis that TNF activates SREBP1 through caspase2 (Casp2) and this can lead to elevated *de novo* lipogenesis in liver. Casp2 is highly activated in HFD-fed *MUP-uPA* mice, which develop NASH, and its activation is largely reduced upon TNFR1-ablation (i.e. in *Tnfr1^{-/-}MUP-uPA* mice). Moreover, activation of SREBP1 in HFD-fed *MUP-uPA* mice is completely blocked in *Casp2*-ablated *MUP-uPA* mice (*Casp2^{-/-}MUP-uPA*), suggesting that Casp2 cleaves/activates SREBP1 in response to TNFR1 signaling in HFD-fed *MUP-uPA* mice. Noteworthy, we found that Casp2 is also activated in livers of patients with NASH but not in those with milder non-alcoholic fatty liver disease (NAFLD), suggesting that Casp2 plays a significant role in the pathogenesis of inflammation-associated metabolic diseases. To better understand Casp2 function, we will use genetically engineered mice, molecular biological studies, and metabolic studies to investigate the mechanism of SREBP1 activation by Casp2 and evaluate the mechanism and pathogenic impact of Casp2-mediated SREBP1 activation in NASH progression and exacerbation of liver metabolic complications. This study will provide a new insights to the molecular mechanisms by which TNF signaling stimulates SREBP1 processing via Casp2, leading to aberrant lipid accumulation during NASH progression and offer new opportunities for development of drugs that ameliorate the pathogenesis of NASH, one of the most severe outcomes of the metabolic syndrome and type 2 diabetes, cardiovascular diseases.

51. Leukotriene D₄ and prostaglandin E₂ synergism in inflammation and asthma

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Rationale: Cysteinyl leukotrienes (LTs-C₄, D₄, E₄) and prostaglandin E₂ (PGE₂) are the metabolites of arachidonic acid, which were shown to be generated at the site of inflammation. However it is not known if there is a cross-talk exists between these two classes of inflammatory mediators. **Methods:** We induced vascular (ear) inflammation by injecting agonists into mouse ear and assessed it by measuring ear thickness and histology. Pulmonary inflammation was examined by sensitization and challenge of *Dermatophagoides Farinae* (Der f) (3 ug) intranasally in the presence or absence of LTD₄+PGE₂ and the inflammation was analyzed by histology. The activation of signaling molecules were measured by their expression and phosphorylation by Immuno-blotting and by RT-PCR. PGD₂ and MIP1β generation was measured by ELISA. **Results:** LTD₄+PGE₂ potentiated both vascular permeability and edema, gearing the system towards pro-inflammation in WT mice and not in *Kit^{W^{-sh}}* mice. Further, LTD₄+PGE₂, via CysLT₁R and EP₃, enhanced Erk and c-fos phosphorylation, inflammatory gene expression, MIP1β secretion, cyclooxygenase-2 (COX-2) up-regulation and PGD₂ generation in MCs. Interestingly, we found that this synergism is mediated through Gi, PKG, and Erk signaling. LTD₄+PGE₂ potentiated effects are partially sensitive to CysLT₁R or EP₃ antagonists, but are completely abolished by simultaneous treatment of both *in vitro* and *in vivo*. Importantly, LTD₄+PGE₂ potentiated Derf-challenged pulmonary inflammation as determined by enhanced cellular infiltration, mucous production and expression of Gob 5, MUC5AC and IL-13 transcripts in lung. **Conclusions:** Taken together, our findings unravel a unique LTD₄-PGE₂ interaction impacting MCs via CysLT₁R and EP₃ involving Gi, PKG and Erk, contributing to inflammation *in vivo*. Our results further suggest that targeting both cysLT₁R and EP₃ has an advantage in attenuating inflammation.

52. Uncovering biologically significant lipid isomers with liquid chromatography, ion mobility spectrometry and mass spectrometry

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Understanding how biological molecules are generated, metabolized and eliminated in living systems is important for interpreting processes such as immune response and disease pathology. While genomic and proteomic studies have provided vast amounts of information over the last several decades, interest in lipidomics has also grown due to improved analytical technologies revealing altered lipid metabolism in type 2 diabetes, cancer, and lipid storage disease. Mass spectrometry (MS) measurements are currently the dominant approach for characterizing the lipidome by providing detailed information on the spatial and temporal composition of lipids. However, interpreting lipids' biological roles is challenging due to the existence of numerous structural and stereoisomers (i.e. distinct acyl chain and double-bond positions), which are often unresolvable using present approaches. Using ion mobility spectrometry (IMS) technology we were able to separate fatty acids based on the double bond locations and orientations in fatty acid isomers, separation in intact lipids based on the sn-positions, cis/trans orientations, as well as R/S hydroxyl groups. Liquid chromatography (LC) IMS-MS separated lipid classes and subclasses. The identification of lipid isomers in complex biological samples was also achieved. Here we show that combining LC-IMS measurement with MS analyses distinguishes intact lipid isomers and allows insight into biological and disease processes.

53. Pharmacokinetics of oxygenated DHA, 4(RS)-4-F4t-Neuroprostane in rodent brain

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Docosahexaenoic acid (DHA) are predominantly abundant in cellular membranes of the central nervous system (CNS). The exact molecular mechanisms of how DHA exert its beneficial roles to the CNS remains largely unexplored, but is associated to the generation of anti-inflammatory enzymatic-derived lipid mediators, such as Resolvins (Rv) D1-6, Neuro-protectin D1 (NPD1/PD1), and Protectin DX (PDX), upon receptor-mediated signal transduction. Imbalance of reactive species in tissues spontaneously triggers non-enzymatic lipid peroxidation, especially the highly unsaturated fatty acid, DHA. Neuroprostanes (NeuroPs) are nonenzymatically derived lipid mediators from DHA oxygenation and are believed to be the gold standard for oxidative damage in the brain. These NeuroPs have a vital role to play in the pathogenesis of neurodegenerative diseases like the Alzheimer's disease, Parkinson's disease, and multiple sclerosis, as the levels of NeuroPs were elevated in the brains of these patients. Not until recently, a 4-series NeuroP (4(RS)-4-F4t-NeuroP), among the eight possible regioisomeric groups has demonstrated to possess anti-arrhythmic property *in cellulo* and *in vivo*. This study, for the first time, has further emphasized that not all isoprostanoids are unfavourable. Also, we identified that 4(RS)-4-F4t-NeuroP was the predominant isoprostanoids in normal pig and rat brain. To further probe on its functionality in the brain and its pharmacokinetics, we infused 4(RS)-4-F4t-NeuroP intravenously in male Sprague Dawley rats. The rats were sacrificed at eight different time points after injections: 0 (control), 5 s, 30 s, 1 h, 2 h, 4 h, 6 h and 24 h. Plasma was separated from the whole blood and organs were harvested immediately. The plasma concentration of 4(RS)-4-F4t-NeuroP was quantified throughout the time-course using the LCMS/MS. Our preliminary data suggest that the administration of 4(RS)-4-F4t-NeuroP is rapid with a fast rate of elimination, similar to those administrated with F2-Isoprostanes as previously reported by others.

54. Evaluation of Phytoprostanes and Phytofurans in different nuts and seeds

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Phytoprostanes (PhytoPs) and phytofurans (PhytoFs) are non-enzymatic lipid peroxidation products of α -linolenic acid (ALA) in plants. Recently, it is discovered that both PhytoPs and PhytoFs are present in some nuts and seeds, which indicate these are readily available in our daily diet. Furthermore, PhytoPs showed some protection on oxidant-induced neuroblastoma cells. To evaluate sources of PhytoPs and PhytoFs from daily food items, we investigated the level of ALA, PhytoPs and PhytoFs in 9 different kinds of common dietary nuts and seeds including almond, peanut, pine nut, walnut, chia seed, flaxseed, sunflower seed, black sesame seed and white sesame seed. The oil was extracted from nuts and seeds by Soxhlet extraction, and analysed by LC-MS/MS. We found the ALA content of seeds in particular chia seed exceeded those of nuts. Further, seeds such as chia seed and flaxseed had significantly high amount of PhytoPs whereas nuts such as almond had the highest amount of PhytoFs. Surprisingly, antioxidant rich walnut had relatively low amount of ALA but a high amount of PhytoPs and PhytoFs. We also tested the antioxidant capacity and phenolic content of these samples by ABTS assay and Folin-Ciocalteu assay respectively. However, we observed no relationship between the level of antioxidant capacity and the level of PhytoPs and PhytoFs, and moreover no relationship between the phenolic content and the level of PhytoPs and PhytoFs were identified. This study showed that PhytoPs and PhytoFs are found commonly in nuts and seeds, and the level of ALA did not necessarily indicate higher PhytoPs and PhytoFs. Further investigation on the health benefits and bioavailability of PhytoPs and PhytoFs are currently being conducted in our laboratory.

55. 'Sequence alignment' inspired comparative analysis tool for lipidomes

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Lipids vary between model organisms and humans. Cholesterol, a major component of mammalian cell membrane is absent in *Drosophila*. Instead, structurally related Ergosterol is the prominent sterol in *Drosophila* and Yeast. Sphingomyelin, also a major component of cell membrane in mammals versus CeramidePhosphoEthanolyamine in *Drosophila* is another example. Organisms choosing different-but-structurally-related lipids to carryout similar biological functions is not a surprise. In spite of compositional differences, model organisms are routinely employed for deciphering lipid metabolic pathways and signaling cascades. Given the rapid advances in mass spectrometry and availability of lipidome data of many model organisms and mammalian tissues, we ask the question, can we compare lipidomes based on lipid-structural-differences and derive a "lipidome homology" measure analogous to "sequence homology" metrics available for amino- and nucleic- acid sequences? We contemplate that "lipidome homology" measure will assist model organism selection and act as a bridge connecting lipidomics and evolutionary biology. We developed computational workflow to programmatically convert lipid structures into sequences and then compare lipid sequences using popular alignment algorithms. Using lipid structural similarity as the basis, we project lipidome(s) in chemical space and measure the extent of overlap between lipidomes. This workflow was tested by projecting all lipids from LIPIDMAPS Structure Database into chemical space. Lipid clusters formed patterns corresponding to classification proposed by LIPIDMAPS consortium. Further, we compared lipidomes of yeast elongase mutants (Ejsing et. al., 2009) and *drosophila* larval tissue lipidomes (Carvalho et. al., 2012) and observed clustering pattern corresponds to phenotype and tissue-type respectively.

Reference:

Marella C, Torda AE, Schwudke D (2015) The LUX Score: A Metric for Lipidome Homology. *PLoS Comput Biol* 11(9): e1004511. doi: 10.1371/journal.pcbi.1004511

56. Macrophage eicosanoid biosynthesis elicited by oxidized phospholipids and oxidized low density lipoprotein

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Atherosclerosis is a chronic inflammatory condition of large and medium-compliance arteries and is considered the principle underlying cause of cardiovascular disease. Atherosclerotic plaques contain lipid-laden macrophage foam cells, which are a major source of proinflammatory factors that directly contribute to disease progression, plaque instability and ultimately acute coronary events. Macrophage engulfment of oxidized low-density lipoprotein (oxLDL) leads to massive accumulation of cholesterol-esters stored within cytosolic lipid droplets. Lipid droplets produced in response to bacterial lipopolysaccharide have been shown to contain the enzymes required for eicosanoid biosynthesis, suggesting that foam cells may be a source of eicosanoids during atherosclerosis. Eicosanoids are lipid signaling molecules that can have powerful effects on the inflammatory microenvironment of atherosclerotic lesions. Furthermore, macrophage expression of key eicosanoid biosynthetic genes has been demonstrated within human plaques. It is widely accepted that foam cell proinflammatory activity is in part elicited by oxLDL. However, little is known about how oxidized lipids and oxLDL alter macrophage eicosanoid production. Our laboratory has demonstrated that the oxidation of human LDL leads to the formation of 1-palmitoyl, 2-oxovaleryl phosphatidylcholine (POVPC), an oxidized phospholipid. Furthermore, POVPC forms Schiff bases with lysine residues of the LDL apoprotein. Such products are recognized by macrophage receptors including CD36 and are found in plaques from humans with coronary artery disease. We have used lipidomic approaches to quantitate time dependent eicosanoid biosynthesis by macrophages exposed *in vitro* to purified oxidized phospholipids, synthetic POVPC-peptide or oxLDL. Our results suggest POVPC-peptide alone only weakly activates eicosanoid biosynthesis, but does elicit a priming effect whereby secondary exposure to a proinflammatory stimulant (ATP) leads to an enhanced production rate of cyclooxygenase-derived metabolites. Results with oxLDL will also be discussed. These studies will further our understanding of how oxidized lipids influence macrophage activation and eicosanoid metabolism and their potential implication for the pathogenesis of atherosclerosis.

57. Inhibition of TRPV4 mediated signaling decreases TGF- β 1 induced fibroblast differentiation and ameliorates house dust mite induced asthma in mice *

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Asthma is a chronic progressive lung disease characterized by airway inflammation and lung tissue fibrosis. Key pathological events that occur during tissue fibrosis include fibroblast differentiation to contractile myofibroblasts, fibroblast proliferation, and increased synthesis and accumulation of collagen and other extracellular matrix (ECM) proteins. Fibrosis in the lung tissue results in airway remodeling and increased stiffness, compromising organ function. Transient receptor potential vanilloid 4 (TRPV4), a mechanosensitive ion channel expressed in lung fibroblasts, responds to changes in ECM stiffness and mechanical forces. Previous studies have shown that, TRPV4 plays a role in the differentiation of cardiac fibroblasts to myofibroblasts. In the present study, we show that TRPV4 mediates lung fibroblast differentiation and aim to elucidate the signaling mechanism(s) involved. We found that TRPV4 was functionally expressed in normal human lung fibroblasts (NHLF) and that knocking-down TRPV4 inhibited TGF- β 1 induced fibroblast differentiation, as determined by decreased α -SMA expression. Similarly, TRPV4 antagonists AB159908 (AB1) and RN1734 (RN) also significantly inhibited TGF- β 1 induced expression of α -SMA and fibronectin protein, as well as pro-fibrotic genes SM22, Collagen1A1, and MRTF-A. We also compared TGF- β 1 induced expression levels of these fibrotic markers in NHLF and diseased human lung fibroblasts (DHLF) and found elevated levels in DHLF. To confirm the role of TRPV4 in lung fibroblast differentiation *in vivo*, we employed a house dust mite (HDM) induced asthma model in wild-type (WT) and TRPV4 knock-out (TRPV4 KO) mice. Following exposure to HDM, histological analysis of lung tissue and bronchoalveolar lavage fluid (BALF) revealed airway thickening, collagen deposition, goblet cell accumulation, and an increase in the inflammatory response in WT mice. Interestingly, we observed that absence of TRPV4 showed markedly reduced airway remodeling and decreased inflammatory response. Altogether, these findings highlight a major role for TRPV4 in lung fibroblast differentiation and HDM induced asthma in mice.

58. Fatty acid elongation is required for human cytomegalovirus infection

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Host cellular metabolic and lipid biosynthesis hijacking by human cytomegalovirus (HCMV) is required for virus replication. HCMV is a β -herpes virus that is wide-spread, infecting a majority of the global population. Infection is usually asymptomatic; however, pre-natal infection or infection of immunocompromised individuals may cause a wide-range of clinical illness including fever, hearing loss (in the case of pre-natal infection), and, in some cases, may threaten the life the infected individual. HCMV has been associated with neuroblastomas, cardiovascular disease and age-associated immune dysregulation. HCMV, like all viruses, are cellular parasites. Instead of encoding its own metabolic machinery HCMV “steals” metabolites including cellular lipids to build its envelope. Previous systems-level examination of metabolites and their fluxes found that HCMV notably increased the flow of carbon from glucose to fatty acids. Using LC-MS we found that HCMV dramatically increased very-long fatty acid tails (VLCFAs), specifically those that have 26 or more carbons. Isotopic metabolic tracers revealed that fatty acid elongation, not necessary *de novo* synthesis, was robustly increased by HCMV. We demonstrated that fatty acid elongase 7 (ELOVL7) produces saturated VLCFAs that are required for a function viral envelope. Additionally, ELOVL5 is required for proper virus replication. During infection ELOVL5 produces unsaturated VLCFAs. The unsaturated VLCFAs do not appear to play a role in the virus envelope but instead may mitigate the cellular response to viral metabolic hijacking to ensure a cellular state that favors virus replication. Overall, our findings demonstrate that HCMV institutes a specific lipid metabolism program that generates a unique lipid environment required for viral replication.

59. Torpedo electric organ lipid composition: Toward a functional nicotinic acetylcholine receptor detergent complex

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This study compares the lipid composition, including individual phospholipid molecular species of solubilized nAChR detergent complexes (nAChR-DCs) with those of the bulk lipids from their source, *Torpedo californica* (*Tc*) electric tissue. The analysis of phospholipid molecular species was carried out by Ultra Performance Liquid Chromatography coupled to electrospray ionization mass spectrometry. This lipidomic analysis revealed seventy-seven (77) phospholipid species in the *Tc* tissue. Analysis of affinity purified nAChR-DCs prepared with C-12 to C-16 phospholipid analog detergents alkylphosphocholine (FC) and lysophosphocholine (LFC) demonstrated that nAChR-DCs prepared with FC12, LFC14 and LFC16 contained >60 phospholipids/nAChR, which was more than twice of those prepared with FC14, FC16, and LFC12. Significantly, all the nAChR-DCs lacked ethanolamine and anionic phospholipids, contained only four cholesterol molecules, and a limited number of phospholipid molecular species per nAChR. Preservation of the hydrophobic, membrane-spanning region of the nAChR, are required to maintain the functional conformation. Detergents will disrupt this highly sensitive lipid-protein interface. In order to investigate the possible delipidation effect of the above mentioned detergents, we performed a functional assessment of these nAChR-DCs in *Xenopus* oocytes using a two electrode voltage clamp (TEVC). Upon incorporation into oocytes, only FC12 is similar to the crude, whereas LFC14 and LFC16 nAChR-DCs displayed an increased functionality as compared to the crude *Tc* membrane. All three nAChR-DC displayed different degrees of alterations in macroscopic activation and desensitization kinetics. Our results provide information on the lipidic basis for the functionality and stability of nAChR-DCs. Moreover, these findings will provide a lipidomic strategy to prepare nAChR-DCs suitable for structural studies.

60. Using metabolomics to improve our understanding of asthma

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Background: Asthma is complex lung disease caused by both genetic and environmental factors that affects up to 10% of individuals in developed countries. It is characterized by recurring inflammation and narrowing of the airways which can lead to hospitalization. Asthma consists of a number of distinct phenotypes based on unique clinical characteristics. Treatment is often performed by observing an individual's response to therapy rather than providing a targeted treatment based on specific disease markers. We therefore sought to investigate changes in the metabolome due to the onset of asthma. **Methods:** The primary allergen challenge mouse model which mimics the initial onset of asthma was utilized. C57BL/6 mice were sensitized to ovalbumin (OVA) intraperitoneally on day 0 and 14 and were subsequently challenged with nebulized OVA on days 28, 29, and 30. Matched bronchoalveolar lavage fluid and plasma were collected at 6h, 24h, and 48h from three groups of mice (n=3-5/group) who were sensitized and/or challenged. Samples underwent liquid-liquid extraction followed by liquid chromatography mass spectrometry-based metabolomics. **Results:** Distinct differences were observed among the mice that were OVA/OVA sensitized and challenged compared to the mice that were sensitized or challenged with 398 and 368 dysregulated metabolites in the BAL fluid and plasma respectively. The dysregulated metabolites mapped to four significant, interconnected metabolomic pathways including sphingolipid metabolism ($p=6.6 \times 10^{-5}$), arginine and proline metabolism ($p=1.12 \times 10^{-7}$), glycerophospholipid metabolism ($p=1.3 \times 10^{-10}$), and neurotrophin signaling pathway ($p=7.0 \times 10^{-6}$). **Conclusion:** Although 34% of metabolites overlapped between BAL and plasma, some of the dysregulated metabolites did not follow the same directional trends in both biofluids signifying the potential for studying downstream effects of metabolites in the blood compared to direct effects in the lungs. Our findings point to potential molecular targets which can be used to investigate treatment alternatives in this mouse model, and used in future translational studies in humans.

61. Curcumin mediated apoptosis in human neuroblastoma cells via ROS and sphingolipid generation

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Background: Neuroblastoma is the most common solid tumor of infancy and third leading cause of cancer death in children. Curcumin induces apoptosis and inhibits proliferation, angiogenesis, invasion and metastasis in many human cancer cells. The mechanism of cytotoxicity in neuroblastoma is unclear. **Objectives:** In this study we investigated the effects of curcumin on SMS-KCNR and CHLA-20 human neuroblastoma cells. **Methods:** Cell viability was determined by Alamar Blue assays. Western Blotting was performed to examine downstream signaling pathways. Measurement of endogenous sphingolipids was performed by LC/MS. Sphingolipid pathway enzyme activities were also determined. **Results:** Curcumin was cytotoxic to both cell lines. PARP cleavage was noted at 24 hours, but cleavage of caspases 3, 8 and 9 was not observed. Treatment with the pancaspase inhibitor z-VAD did not reverse cytotoxicity, indicating that curcumin's effects were caspase-independent. LC/MS measurement of endogenous sphingolipids was performed and showed increases in both dihydroceramides and ceramides. Dihydroceramide desaturase (DEGS-1) was inhibited *in-situ* in a dose dependent manner in SMS-KCNR cells. Next, the mechanism of ceramide generation was investigated by measuring the activity of sphingomyelin synthase (SMS) glycosylceramide synthase (GCS), acid ceramidase, neutral ceramidase, acid sphingomyelinase and neutral sphingomyelinase (SMase). At 6hrs, curcumin downregulated SMS activity by 30% and 54% GCS activity by 40% and 42% at concentrations of 10 and 20uM respectively. Curcumin has been demonstrated to induce ROS generation. Pre-treatment with the antioxidants N-acetylcysteine or glutathione abrogated curcumin mediated apoptosis and sphingolipid generation in SMS-KCNR cells. Furthermore, curcumin mediated SMS and GCS inhibition was blocked by these antioxidants. **Conclusions:** ROS plays a key role in sphingolipid and curcumin induced-cytotoxicity in neuroblastoma cells. Modulation of sphingolipid signaling pathways may provide a more effective and novel approach for the treatment of pediatric solid tumors. Curcumin is a potential novel therapy for neuroblastoma.

62. Comparative study: The MALDI-TOF Mass Spectrometry Imaging and LC-QTRAP analysis of lipid profile in different organs from healthy mice at three different ages.

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The MALDI-TOF mass spectrometry imaging is a promising new approach to detect biomolecules and to collect detailed chemical information of the uppermost molecular layers from organs cross sections. Actually, this technique allows direct analysis of intact tissues surface without any prior pretreatment or sample preparation. This tool has the advantage of visualizing the spatial distribution of biomolecules such as lipids and peptides. Furthermore it provides a relative quantification of lipids as well as peptides on adjacent tissue sections. Nevertheless, there is a sensitivity restriction according to the sample. In fact the biological distribution of the tissue could decrease the sensitivity. In this work, the spatial distributions of various specific lipids in freeze-dried mouse organs sections at different ages were monitored by the Maldi-TOF mass spectrometer (MALDI-TOF-IMS). The results were compared to a quantitative study of the total lipid profile of the same organs using LC-MRM approach with an API 5500 LC-Q-TRAP. Both techniques were found to be complementary for the lipid profile study of the different organs.

63. LTC₄ is the major trigger of oxidative DNA damage elicited by ER stress and by cytotoxic agents

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Endoplasmic reticulum (ER) stress and major chemotherapeutic agents damage DNA by generating reactive oxygen species (ROS). Here we show that ER stress and major chemotherapeutic agents induce leukotriene C₄ (LTC₄) biosynthesis in cells of non-hematopoietic lineage. Following ER stress or chemotherapy, an LTC₄ biosynthetic machinery, consisting of microsomal glutathione-S-transferase 2 (MGST2), 5-lipoxygenase, 5-lipoxygenase-activating protein and cytoplasmic phospholipase A2, was activated by assembly at the nuclear envelope. ER stress and chemotherapy also triggered nuclear translocation of the two LTC₄ receptors. Acting in an intracrine manner, LTC₄ then elicits nuclear translocation of NADPH oxidase 4 (NOX4), ROS accumulation, oxidative DNA damage and dsDNA breaks. *Mgst2* deficiency, RNAi and LTC₄ receptor antagonists abolished ER stress- and chemotherapy-induced ROS accumulation and DNA damage *in vitro* and in mouse kidneys. Cell death and mouse morbidity were also significantly attenuated. Hence, MGST2-generated LTC₄ is the major mediator of ER stress- and chemotherapy-triggered oxidative stress and oxidative DNA damage, thereby augmenting cell death. Tumor cells of hematopoietic origin do not express MGST2. Indeed, they remained refractive to chemotherapy in the presence of LTC₄ inhibitors. We therefore propose that LTC₄ inhibitors, commonly used for the treatment of asthma, may alleviate chemotherapy-associated morbidities when used in hematologic malignancies. Furthermore, NOX4 has been implicated in additional major human pathologies, including metabolic diseases, neurodegeneration and osteoporosis. Therefore, inhibition of its activation by approved LTC₄ receptor antagonists may be of even a broader clinical significance.

64. An improved extraction method for determination of prostaglandins in media to investigate up-regulation of prostaglandin in aorta and mesenteric arteries of spontaneously hypertensive rats

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Endothelial dysfunction is an important hallmark of cardiovascular disease such as hypertension. It is well-known that endothelial dysfunction in spontaneously hypertensive rats (SHRs) involves up-regulation of cyclooxygenase (COX)-derived vasoconstrictor prostanoids, which are a subclass of eicosanoids consisting of prostaglandins. Measurement of prostaglandins (PG) in the vasculature is extremely challenging due to the short half-life, stability and matrix effect, thus it remains unclear which prostaglandins are upregulated in the vessels of SHR. Therefore, the aim of the study is to improve extraction of prostaglandins in media to characterise which prostaglandins are altered in the aorta and mesenteric arteries of SHR. To investigate the level of PG in the vasculature, segments of aorta and mesenteric arteries isolated from male rats, are incubated in physiological saline buffer for 1 hour at 37°C. At the end of the incubation period, the endothelium-dependent agonist, 10 µM of acetylcholine was applied to stimulate the production of prostaglandins for 10 min. The vessel segments were removed and the buffer collected and snapped frozen in liquid nitrogen and stored at -80°C for further analysis. Prostaglandins in media was extracted using hexane and ethyl acetate, then analysed using Liquid Chromatography Mass Spectrometry (LC/MS). The recovery is improved using biphasic extraction and the analysis has shown upregulation of few prostaglandins species in mesenteric and aortae arteries of SHR.

65. Regulation of arachidonic acid metabolism by PLA2 enzymes in cancer

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The metabolic networks that mediate and regulate the inflammatory response in cancer are complex and how they promote cancer is still poorly defined. The eicosanoid lipid network, a key mediator of the induction and resolution of inflammation, is inappropriately regulated in the development and progression of cancer. Over the last decade, we and others have established that aberrant expression and activation of enzymes that control arachidonic acid flux through these pathways and, in particular, two phospholipase A₂ (PLA₂) enzymes, a secreted PLA₂ (Group IIA PLA₂, hGIIA) and an intracellular PLA₂ (Group IVA PLA₂, cPLA- α) contribute significantly to the promotion of tumour growth in several cancers. Further, pharmacological blockade of these enzymes slows tumour growth in a variety of animal models of cancer (1, 2). In recent years, we have explored the “upstream” and “downstream” pathways that contribute to PLA₂-mediated promotion of tumour cell growth and the mechanisms by which PLA₂ inhibitors block these effects (3-5). Taken together our data suggests that evaluation of the benefit of intervention in the eicosanoid network at these enzymes in cancer is warranted.

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66. The role of oxidized phospholipid-derived lipid mediators in IgE-mediated mast cell activation

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Oxidized phospholipids are generated through reactive oxygen species or enzymatic reactions. Although various oxidized phospholipids have been identified in cell membranes, their physiological functions and production control mechanism are not well understood. Intracellular type II platelet-activating factor acetylhydrolase (PAF-AH (II)) is a monomeric 40-kDa enzyme that was originally identified as an enzyme hydrolyzing the *sn*-2 acetyl group of platelet-activating factor. Unlike other intracellular phospholipase A₂, PAF-AH (II) cannot hydrolyze long fatty acyl chains but can hydrolyze oxidized fatty acyl chains attached to phospholipids. However, the physiological role of PAF-AH (II), an oxidized phospholipid-selective phospholipase A₂, remains to be elucidated. Here we show that suppression of PAF-AH (II) impairs IgE-mediated mast cell activation. Immunohistochemistry analysis revealed that PAF-AH (II) localized with toluidine blue-positive dermal mast cells. PAF-AH (II) knockout (*Pafah2*^{-/-}) mice show markedly reduced passive cutaneous anaphylaxis (PCA) induced by IgE and antigen. Bone marrow-derived cultured mast cells (BMMCs) obtained from *Pafah2*^{-/-} mice appeared normal but displayed a reduction in antigen-induced degranulation. Lipidomics analysis of mast cells revealed dramatic reduction of some oxidized ω 3 fatty acids in *Pafah2*^{-/-} BMMCs. Treatment of *Pafah2*^{-/-} mice with these oxidized ω 3 fatty acids restored IgE-dependent-mast cell activation. Taken together, these results suggest that PAF-AH (II) hydrolyze oxidized ω 3 fatty acids-esterified phospholipids and PAF-AH (II)-derived oxidized ω 3 fatty acids are required for IgE-mediated mast cell activation.

67. Reduction of PIP2 and subsequent normalization of PKC γ activity are involved in the lithium-induced recovery of memory and emotional impairments in DGK β KO mice.

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Binding of ligands including neurotransmitters and hormones to G-protein coupled receptors causes hydrolysis of phosphatidylinositol bisphosphate (PIP2) by phospholipase C, resulting in production of diacylglycerol (DG) and inositol triphosphate. The DG activates protein kinase C (PKC) and it is converted to phosphatidic acid (PA) by DG kinase (DGK). The produced PA regulates activity of several enzymes including mTOR. In other words, DGK plays a pivotal role to control the balance of two important lipid messengers, DG and PA. Among 10 mammalian DGK subtypes, DGK β is enriched in neuron. We have developed DGK β knockout (KO) mice to investigate function of DGK β in nervous system. The KO mice showed memory loss and mania-like behavior but both impairments were rescued by lithium treatment for 10 days. Here, we investigated the molecular mechanism underlying the lithium-induced recovery of the impairments. We compared spine density in the cortex of WT and KO mice before and after lithium treatment, and found that spine density in the KO mice was lower than WT and rescued by lithium treatment. Next, we measured amount of PA by mass spectrometry because PA-mTOR pathway is involved in the DGK β -mediated morphological change of neurons. However there was no significant change in PA level before and after lithium treatment. Instead, lithium treatment significantly reduced almost all types of PIP2 level, suggesting decrease of DG and normalization of PKC activity in the lithium-treated KO mice. Indeed, auto-phosphorylation level of PKC γ was significantly upregulated in the KO mice and normalized by lithium treatment. Furthermore, lithium-treatment or PKC γ inhibition rescued morphological impairment of primary-cultured cortex neurons from the KO mice. These results indicate that reduction of PIP2 and subsequent inactivation of PKC γ normalizes morphology of neurons, contributing to the lithium-induced recovery of memory and emotional impairments in DGK β KO mice.

68. Regulation of mRNA eicosanoid enzymes metabolism expression in bone marrow-derived macrophages activated by classical and alternative pathway

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The innate immune response is dependent on the interaction of cells with certain molecules of pathogens and stimulation by cytokines of the pattern of response of T lymphocytes (Th1 or Th2). Classical macrophage activated (M1) by IFN- γ is fundamental in the immune response against intracellular microorganisms and tumors. However, alternative macrophage activated (M2) with IL-4 /IL-13, plays a role in promoting tissue repair and remodeling. Macrophages type M1 and M2 are not only distinguished about the function in the body, but also by different expressions of receptors and enzymes related to cell metabolism. Herein, we used cells isolated from C57BL/6 mice and cultured with M-CSF to generate bone marrow-derived macrophage (BMDM). Also, qRT-PCR to determine mRNA expression for phenotypes markers, receptors and enzymes involved on eicosanoid pathway in polarized macrophages. We demonstrated that priming macrophage with IL-4 was important to up-regulate mRNA expression for Arginase-1, YM1 and FIZZ1 markers described for M2, and non-effect on iNOs expression modulation. For eicosanoids enzymes metabolism, we observed a time-dependent mRNA expression for polarized BMDM, an earlier (2 to 6 hours after treatment) up-regulation for 5-LO, FLAP, LTA4H in both M1 and M2, and increased expression of LTC4-synthase on M2. However, we did not observed mRNA expression of 12-LO and 15-LO in those BMDM. For prostaglandins metabolism, we observed an up-regulation (24 hours) for PGD2-synthase and COX-2 mRNA in M1 and M2 macrophage, but more prominent in M1. The pattern of eicosanoid receptors was different in M1 and M2. The M1 expressed more BLT1 and EP3, and M2 expressed more EP2, EP4 and BLT2 mRNA. In conclusion, we speculated that plasticity of macrophage in M1 and M2 affect the lipid metabolism, providing useful information about the pathological mechanisms in which those cells participate.

69. Subcellular localization of a 2-arachidonoyl glycerol signaling cassette in developing retinal ganglion cell axons is consistent with formation of hotspots

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Objective: Endocannabinoids (eCBs) modulate axon growth in glutamatergic projection neurons, but their role in retinal ganglion cells (RGCs) is poorly understood. We investigated whether RGCs exhibit an eCB system topology consistent with formation of 2-arachidonoyl glycerol (2-AG)-enriched hotspots in an embryonic retinal explant model. **Methods:** Explants were stained for monoacylglycerol lipase (MGL), diacylglycerol lipase α (DGL α), and cannabinoid receptor type 1 (CB1R) and imaged by confocal microscopy. Images were segmented into regions of interest (ROIs) including growth cone (GC) central and peripheral domains (CDs and PDs) and 5 μ m segments of axon. Gray value for each ROI was recorded and normalized to the CD. **Results:** CDs showed maximal expression for MGL and DGL α , while PDs stained lightly. Moving proximally from CDs along MGL-stained processes, expression was stable in the distal 35 μ m of axon then tapered down slowly, while DGL α tapered down rapidly from the CD through the first segments of distal axon. CB1R signal complemented DGL α , rapidly tapering up >2-fold in the same region. CB1R antagonist O-2050 enhanced MGL expression in an MGL inhibitor JZL184-sensitive manner, while JZL184 alone reduced MGL expression. **Discussion:** Previous studies of non-RGC glutamatergic neurons demonstrated exclusion of MGL from the GC, where DGL α was enriched. Putative 2-AG "hotspots" may spatially restrict 2-AG signaling competence. Our model of RGC axon growth shows differential expression of MGL, DGL α , and CB1R in the distal axon and GC that is distinct from the previously reported pattern but consistent with the hotspot hypothesis. This arrangement should favor enhanced 2-AG tone in the distal axon and GC that leads CB1R expression. We also find that axonal MGL expression is suppressed by 2-AG tone at CB1R. Forebrain projection target-derived 2-AG might coordinate synaptogenesis with acquisition of mature eCB system topology through related mechanisms. This work is supported by Glaucoma Research Foundation.

70. A mouse sphingolipids atlas and its use in translational research

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Murine models are widely used to study mammalian biology. Lipids are crucial metabolites for healthy functioning of any organism. Sphingolipids are important components of the membranes (structural function) and also regulate various cellular activities (signaling function). Any disruption in the sphingolipid metabolism, enzymes or pathway can trigger pathological processes. We are investigating (1) the sphingolipid composition of different murine tissues to create a reference atlas of the sphingolipidome in wild type C57BL/6 mice; (2) the possible link between sphingolipid profiles and tissue functions; (3) any gender-related difference in the murine sphingolipidome; (4) the genetic basis for the differences in the sphingolipidome in different tissues; (5) the perturbation of the sphingolipidome in pathological conditions that affect specific organs to find sphingolipid species that could be used as biomarkers or to clarify the mechanism of these pathologies; (6) the reliability of our mouse atlas as a reference for studies on disease models. To define our atlas and to detect variations of the sphingolipidome in different conditions, a comprehensive list of sphingolipids (~300 molecular species), including ceramides, sphingomyelins, glycosphingolipids, sphingoid bases and sphingosine phosphate(s) will be quantified by targeted LC-MS in all the samples examined.

71. Lipidomic analysis in Amyotrophic Lateral Sclerosis (ALS): looking for footprints of disease onset and progression

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Amyotrophic Lateral Sclerosis (ALS) is a fatal motor neuron degenerative disorder with no effective therapy available. The lack of current treatments for ALS or early diagnosis highlight the absence of a comprehensive understanding of the biological mechanisms underlying the changes that occur during the process of neurodegeneration. A blood-based biomarker could act as a screening tool to identify at-risk individuals. We are focusing on small-to-medium molecules as final products of inflammatory processes associated with onset of the disease. Analysis is focused in products derived from arachidonic acid (AA) metabolism by the lipoxygenase and cyclooxygenase pathways. Preliminary results on G93ASOD mice showed different behavior of lipoxygenase derived products. An increase in 12-HETE levels, when clinical symptoms appeared, was observed without changes in wt mice. In addition, 5-HETE levels decreased faster in G93ASOD mice than wt suggesting alterations in lipoxygenase pathway. This is in agreement with reports about formation and/or inhibition of 12/15-LOX in central nervous system, proposing that 12-HETE may be involved in oxidative damage in brain. Prostaglandins E₂ and D₂ play key roles in pathophysiological processes in brain, including modulation of synaptic plasticity and neuroinflammation. When analyzed, both products exhibited similar behaviors in both wt and G93A mice. No changes in prostaglandins levels were observed in the plasma of transgenic mice while wt levels were greater at day 120. In addition, preliminary analysis of plasma samples from both wt and G93ASOD mice showed quantitative differences in lipid-derived products, which correlated with disease onset and progression. Overall, we are proposing key mediators of AA-derived pathways as novel footprints of ALS onset and progression. Finally, lipidomic analysis are being performed in ALS patients with the aim to discover new molecules that could diagnose ALS patients from controls improving our current knowledge of molecules associated with ALS and their underlying biology.

72. Defining the binding mode of a potent inhibitor in the active site of lipoprotein associated phospholipase A₂

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Phospholipase A₂ constitutes a superfamily of enzymes that catalyzes the hydrolysis of phospholipid substrates at the *sn*-2 position. Group VIIA lipoprotein associated phospholipase A₂ (Lp-PLA₂), also known as platelet-activating factor acetyl hydrolase (PAF-AH), is known to be associated with LDL and HDL (*Chem. Rev.* **2011**, *111*, 6130-6185). Lp-PLA₂ specifically releases oxidized and very short chain fatty acids. Previous DXMS studies and X-ray crystal structures have provided insight into the interaction of Lp-PLA₂ with phospholipid vesicles (*J. Biol. Chem.* **2008**, *283*, 31617-31624). Hydrogen Deuterium Exchange Mass Spectrometry (DXMS) studies suggested that Lp-PLA₂ interacts with membrane phospholipids (*Biochemistry.* **2011**, *50*, 5314-5321) as well as with apo A1 and at an additional location with intact HDL (*J. Lipid Res.* **2013**, *54*, 127-133). The peptide regions of Lp-PLA₂ found to interact with membranes were used to insert the enzyme in a membrane patch. This system was subjected to minimization, equilibration and molecular dynamics (MD) simulations using NAMD (*J. Comput. Chem.* **2005**, *26*, 1781-1802). Clustering analysis allowed us to identify different conformations of Lp-PLA₂ suitable for docking calculations. DXMS binding studies were employed to identify the peptide regions of the active site that interact with a potent and specific inhibitor GSK SB-402564. These regions were used to define the binding site during the docking calculation and a 3D enzyme-inhibitor complex was generated revealing a detailed binding mode of the inhibitor. MD simulations of the complex in the presence of the inhibitor allowed us to identify interactions of the inhibitor with the Lp-PLA₂ binding site and conformational changes that occur upon binding. This is the first detailed study on the binding mode of this Lp-PLA₂ inhibitor using HD exchange data. This complex provides insight into the inhibition mechanism of an Lp-PLA₂ inhibitor.

73. Molecular species of phospholipids in the brain of *Abcd1*-deficient mice

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X-linked adrenoleukodystrophy (X-ALD) is an inherited peroxisomal disorder that displays a neurological phenotype such as cerebral demyelination. X-ALD is caused by a mutation in ABCD1 gene that is critical for the transport of very long chain fatty acids (VLCFAs) into peroxisomes for their beta-oxidation. It has been shown that VLCFAs and VLCFA-containing lipids are accumulated in plasma and fibroblasts from X-ALD patients, however, the profile of VLCFAs and VLCFA-containing lipids has not been fully characterized in the central nervous system. In this study, we examined brain phospholipids in *Abcd1*-deficient mice by liquid chromatography-mass spectrometry (LC-MS) analysis. Firstly, each phospholipid species was quantified by multiple reaction monitoring in the positive ion mode. Secondly, fatty acids in phosphatidylcholine and sphingomyeline that are significantly increased or decreased in *Abcd1*-deficient mice brain were examined by MS/MS/MS analysis. VLCFA-containing phosphatidylcholine and sphingomyeline that have more than 22 carbons are increased in *Abcd1*-deficient mice. Notably, we have confirmed that VLCFAs are mainly saturated or mono-unsaturated. Moreover, we found that VLCFAs are mainly in the *sn*-1 position of PC and PE. These results indicate the molecular machinery responsible for the incorporation of VLCFA in the *sn*-1 position of phospholipids as well as the selective production of saturated or mono-unsaturated VLCFAs

