3rd Annual Northern Great Plains Lipids Conference

June 3-4, 2017

St. Boniface Hospital Albrechtsen Research Center, Winnipeg, Manitoba, Canada

Program

https://ngplc.blog/

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Lipids
3rd Annual Northern Great Plains Lipids Conference Program
St. Boniface Hospital Albrechtsen Research Center

Saturday, June 3, 2017

Time
7:30-8:45 Breakfast (muffins, tea biscuits, tea, coffee and juice) & Poster Setup
8:45 Welcome: Grant Hatch
8:50-10:30 Lipid School-Lipidomics/Lipid Metabolomics (Chair – James House)
8:50-9:40 Amir Ravandi
Application Of Lipidomics To Human Pathology
9:40-10:30 Michel Aliani
Lipidomics Workflow For Food And Biological Samples
10:30-10:50 Coffee Break & Poster Viewing
10:50-12:20 Nutrigenomics & Nutrigenetics (Chair – Peter Jones)
10:50-11:50 Plenary Speaker, Marie-Claude Vohl
Nutrigenomics And Nutrigenetics Of The Metabolic Response To An Omega-3 Fatty Acid Supplementation
11:50-12:20 Peter Jones
Common Single Nucleotide Polymorphisms Involved In Lipid Metabolism Fail to Modulate Triglyceride-Lowering Effect Of DHA, But Some May Alter Other Lipid Parameters: Results From A Clinical Randomized Controlled Feeding Trial In Adults With Metabolic Syndrome
12:20-1:30 Lunch (Sandwiches, drinks) & Poster Viewing
1:30-3:30 Plant Lipids (Chair – Eric Murphy)
1:30-2:30 Plenary Speaker, Brent Hulke
Understanding Genetic Stability Of Fatty Acid Profiles Over Contrasting Environments For Improvement Of Oilseed Sunflower
2:30-3:00 Guanquin (Gavin) Chen

Structure-Function Relationship In Recombinant Plant DGAT1 Variants With Increased Performance

3:00-3:30 Susan Raatz

Modeling The Effects Of High Oleic Oil Replacement On Fatty Acid Intake In The Diet Of US Adults

3:30-4:30 Coffee Break & Poster Viewing

4:30-6:10 Trainee Oral Presentations (Chair – Matthew Picklo)

4:30-4:50 Youjia Du

Docosahexaenoic Acid (DHA) Activation Of P38MAPK In Endothelial Cells Is Dependent On Growth State

4:50-5:10 Christopher D. Pascoe

Intranasal Simvastatin Prevents Allergen Challenge-Induced Changes In Lipid Metabolites

5:10-5:30 Samantha D. Pauls

Oxylipins Derived From The Plant Omega-3 Lipid α-Linoleic Acid Can Directly Modulate Macrophage Function

5:30-5:50 Aleksandra Stamenkovic

Adult Rat Cardiomyocyte Cell Death Induced By Oxidized Phosphatidylcholines (OxPC) Through The Ferroptotic Pathway During Ischemia/Reperfusion

5:50-6:10 Stephanie M. Kereliuk

Early-Life Exposure To Gestational Diabetes Alters Mitochondrial Bioenergetics And Cardiac Function In Rat Offspring

7:00-10:00 Conference Reception

The Pint, 274 Gary Street
3rd Annual Northern Great Plains Lipids Conference Program

Sunday, June 4, 2017

Time
8:00-9:30  Breakfast (muffins, tea biscuits, tea, coffee and juice)
           & Poster Viewing
9:30-11:30 Lipids & Clinical Outcomes (Chair – Carla Taylor)
            9:30-10:30 Plenary Speaker, William Harris
            Omega-6 And Omega-3 Fatty Acids: Partners In Prevention
            10:30-11:00 Matthew Picklo
            Incorporation Of Dietary N-3 Fatty Acids Into Selective Plasma
            Lipids In Humans After Salmon Intake
            11:00-11:30 Carla Taylor
            LDL-Cholesterol Lowering By Canola Oil In Persons With Mild
            Hypercholesterolemia Is Influenced By Arterial Stiffness
11:30-12:30 Lunch (Finger food, drinks) & Poster Viewing
12:30-2:30 Lipids Signaling & Metabolism (Chair – Susan Raatz)
            12:30-1:30 Plenary Speaker, Douglas Mashek
            Beyond Energy Storage – The Lipid Droplet As A Signaling Node
            That Regulates Cell Function And Metabolism
            1:30-2:00 Grant Hatch
            Expression Of Human Monolysocardiolipin Acyltransferase-1
            Improves Mitochondrial Function In Barth Syndrome Lymphoblasts
            2:00-2:30 Eric Murphy
            The Blood Brain Barrier And Fatty Acid Uptake: Role Of The BBB
            As A Metabolic Barrier
2:30-3:00  Coffee Break & Poster Viewing
3:00-4:40 Trainee Oral Presentations (Chair – Harold Aukema)
            3:00-3:20 Brittany L. Moyce
            Altered Hepatic Fatty Acid And Mitochondrial Metabolism
            Contributes To Development Of Gestational Diabetes Mellitus In
            Pregnant Adiponectin-Deficient Mice
3:20-3:40 Afroza Ferdouse
Dietary Oils And Sex Differentially Alter Rat Heart Oxylipin And Fatty Acid Profiles

3:40-4:00 Victoria Sid
Regulation Of Hepatic Inflammation By Folic Acid In Non-Alcoholic Fatty Liver Disease (NAFLD)

4:00-4:20 Zahra Solati
Identification Of Bioactive Lipids During Renal Ischemia Reperfusion (I/R) Injury

4:20-4:40 Thomas Mahood
Prenyltransferase Expression And Function In Lung Fibroblasts From Moderate COPD Patients

4:40 Conference Conclusion
List of Speaker Abstracts, Saturday, June 3, 2017

Lipids School

Amir Ravandi, University of Manitoba

Dr. Amir Ravandi received his undergraduate degree, from University of Toronto and went on to complete a PhD at the Banting and Best Medical Research Institute at University of Toronto. He was at the Terrence Donnelly Vascular Research Labs at St. Michael's's Hospital as postdoctoral fellow. After obtaining his MD at University of Toronto he went on to complete his internal medicine and cardiology training at McMaster University. He completed a fellowship in coronary and peripheral vascular interventions at University of California at San Diego.

Dr. Ravandi is a clinician scientist at St. Boniface Hospital and Research Director for the Section of Cardiology at University of Manitoba. He is a principal investigator at the Institute of Cardiovascular Sciences. His current focus is utilizing lipidomics to further our understanding of myocardial ischemia and plaque rupture.

APPLICATION OF LIPIDOMICS TO HUMAN PATHOLOGY

The field of lipidomics is largely driven by the novel mass spectrometric technologies that allow for detailed analysis of complex lipid mixtures found in biological material. The biochemical experiments involved in lipidomics begin with extraction of lipids from various samples such as tissues, cells, different organisms, biofluids, etc.

The complex lipid mixture is analyzed in a targeted or non-targeted fashion by one or more analytical techniques (GC-MS, HPLC, LC-MS, etc.) to obtain a lipid profile that contains information on the lipid composition of different classes of lipids such as monoacylglycerols (MAG), diacylglycerols (DAG), triacylglycerols (TAG), free fatty acids (FFA), cholesterol, glycolipids, oxylipins and phospholipids present in the starting material. These individual lipid classes are further analyzed in detail to quantify the abundance of saturated, unsaturated, or oxidative modification.

The general focus of our laboratory is to study lipid oxidation products and their contribution to cardiovascular pathology and inflammation. Our laboratory uses LC/MS/MS Lipidomic platform for quantitation and identification of lipid oxidation products in both animal models and clinical conditions to determine the involvement of oxidized lipids in both cardiac and vascular lesions. The ultimate goal is to not only identify the contribution of bioactive lipids to cardiac muscle injury but also to determine new therapeutic approaches. We have not only focused on cardiovascular lipidomics but have ongoing collaborations investigating lipid changes in Chronic Obstructive Lung Disease, Chemotherapeutic induced cardiomyopathy, Renal reperfusion injury, and lipid markers of major depression.
Lipids School

Michel Aliani, University of Manitoba

Michel Aliani is a Professor at the Department of Human Nutritional Sciences at the University of Manitoba and a member of the Canadian Centre for Agri-Food Research in Health and Medicine in Winnipeg, Manitoba. He was educated in France (B.Sc. and Engineering degree in Agri-Food Biochemistry) and in Northern Ireland (PhD, and Post-doctoral at Queen’s University Belfast) prior to move to University of Manitoba in 2007. His area of scientific expertise includes food science, mass spectrometry and metabolomics.

Research focus and interests

1. To provide the scientific and molecular basis for the development and successful marketing of functional foods targeted to patients as well as healthy populations in the world and

2. To investigate the effect of active compounds on metabolic pathways in animal and human model.

LIPIDOMICS WORKFLOW FOR FOOD AND BIOLOGICAL SAMPLES

The ‘metabolome’ is comprised of the major classes of biological molecules: sugars, amino acids, nucleotides, and lipids. Metabolomics is the scientific study to characterize and identify the metabolome. Several steps may be taken to optimize the metabolomics workflow for any of the four major classes of biological metabolites. The systematic study of the entire lipid profile of a cell/tissue/organ/organism is referred to as “lipidomics”. Lipids are a diversified group of amphipathic molecules involved in functional and structural integrity of cellular pathways. Mass spectrometry is a wildly used technology in metabolomics fields for the identification and quantitation of key metabolites/ lipids in a targeted and/ or non-targeted approach. This workshop is designed to familiarize the audience with practical aspects of lipidomics workflow for MS and MS/MS data preparation and interpretation.
Plenary Speaker

Marie Claude Vohl, Laval University

Marie-Claude Vohl completed her graduate studies at Laval University (1992-1997). She was interested in the genetics of dyslipidemia and obesity-related metabolic complications. In 1997-1998, she was enrolled in a post-doctoral fellow at the University of Ottawa Heart Institute where she studied antioxidant properties of HDL particles. During her second post-doctoral training (1998-1999) at McGill University in Montreal, she was interested in the genetics of complex diseases. She has been appointed as a professor at the Department of Food Science and Nutrition at Laval University (Quebec City) in 1999. Her research projects are aimed at the genetic/epigenetic dissection of the obesity-related metabolic complications. She is also interested in nutrigenetics/nutrigenomics and conducted different cross-sectional and intervention studies examining the combined effects of fish nutrients and genetic factors on cardiometabolic risk factors.

Since the beginning of her career, Professor Marie-Claude Vohl has published more than 220 peer-reviewed papers. She has been invited to give several presentations in national and international conferences. Her research programs are funded by CIHR, HSFC and NSERC. Since 2010, she is Canada Research Chair Tier 1 in Genomics Applied to Nutrition and Health.

NUTRIGENOMICS AND NUTRIGENETICS OF THE METABOLIC RESPONSE TO AN OMEGA-3 FATTY ACID SUPPLEMENTATION.

Although several definitions are existing, for the purpose of this conference, nutrigenetics will be defined as the science interested in the effect of genetic variation on nutrient requirements. Nutrigenomics will then be referred as the science exploring the nutrient impact on gene expression. It is hoped that the integration of OMICS technologies will promote the understanding of the complex nutrient-gene interactions leading to chronic societal diseases such as cardio-metabolic diseases. In this conference, examples will be given on how OMICS technologies have deepened our understanding of mechanisms underlying cardio-metabolic diseases, and how nutrient-gene interactions influence these. Omega-3 fatty acids sometimes described as regulators of gene expression will be used as a model to explore nutrient-gene interaction effects on gene expression profile and cardio-metabolic risk factors. Remaining challenges in the routine application of nutrigenetics/nutrigenomics for chronic disorders will also be addressed.
COMMON SINGLE NUCLEOTIDE POLYMORPHISMS INVOLVED IN LIPID METABOLISM FAIL TO MODULATE TRIGLYCERIDE-LOWERING EFFECT OF DHA, BUT SOME MAY ALTER OTHER LIPID PARAMETERS: RESULTS FROM A CLINICAL RANDOMIZED CONTROLLED FEEDING TRIAL IN ADULTS WITH METABOLIC SYNDROME

Peter JH Jones, Suhad S AbuMweis, Sunil K Panchal

Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, MB, R3T 6C5, Canada, Department of Clinical Nutrition and Dietetics, Faculty of Allied Health Sciences, The Hashemite University, Zarqa, Jordan, Functional Foods Research Group, Institute for Agriculture and the Environment, University of Southern Queensland, Toowoomba, Queensland 4350, Australia

Background: The triglyceride (TG)-lowering effect of long-chain omega-3 fatty acids found in fish oil including docosahexaenoic acid (DHA) are well documented, although with large inter-individual variability. It has been suggested that genetic variations of key enzymes and proteins involved in fatty acid synthesis or lipid metabolism can influence an individual’s response to a supplemental dose of DHA.

Objective: This study was carried out to investigate whether common single nucleotide polymorphisms (SNPs) in genes involved in DHA synthesis and TG metabolism are associated with responsiveness of blood lipids, lipoprotein and apolipoprotein concentration to dietary treatment by DHA supplied in high-oleic canola oil.

Methods: In a randomized, crossover controlled feeding trial, 129 subjects with metabolic syndrome received high-oleic canola oil (HOHO) and high-oleic canola oil supplemented with DHA (HOHO-DHA), each for 4 weeks. Subjects were genotyped for APOE isoforms, and 8 SNPs including FADS1- rs174561, FADS2- rs174583, ELOVL2- rs953413, ELOVL5- rs2397142, CETP- rs5882, SCD1- rs2234970, PPARα- rs6008259, and LIPF- rs814628.

Results: Overall, consumption of HOHO-DHA oil reduced blood concentrations of TG by 24% compared to HOHO oil. This effect of DHA was not only associated with LIPF-rs814628 but for treatment-by-gene interaction (P > 0.05) with the exception of LIPF-rs814628 (P=0.028). Similarly, no treatment-by-gene interactions were evident in the response to other lipids, lipoproteins, and apolipoprotein to DHA supplement with few exceptions (ELOVL5 and total cholesterol – P=0.039; ELOVL5 and apoB – P=0.045).

Conclusion: The TG-lowering effect of supplemental body-weight based dose of DHA was not influenced by genetic variation in APOE, FADS1, FADS2, ELOVL2, ELOVL5, CETP, SCD1, and PPARα. However, LIPF- rs814628 may interact with DHA to modulate blood TG levels. ELOVL5- rs2397142 may interact with DHA to modulate total cholesterol and apoB concentrations. Findings suggest that nutrigenetics may explain some of the responsiveness of circulating lipid levels to dietary DHA intervention.
**Plenary Speaker**

**Brent Hulke, United States Department of Agriculture**

Dr. Brent Hulke is a research geneticist with USDA-ARS Red River Valley Agricultural Research Center in Fargo, ND, whose work focuses on sunflower breeding and quantitative genetics. Of particular interest to him is sunflower oil quality research, mitigating abiotic and biotic stresses of sunflower, and improving breeding methods for hybrid sunflower. He has developed sunflower inbred lines adapted for the United States and Canada, and has collaborated with Canadian scientists to develop a high yielding, mid-oleic oilseed sunflower hybrid for western Canada named “Honeycomb NS”. He received a PhD from University of Minnesota, Twin Cities, a M. Sc. from Iowa State University, and a B.Sc. from South Dakota State University.

**UNDERSTANDING GENETIC STABILITY OF FATTY ACID PROFILES OVER CONTRASTING ENVIRONMENTS FOR IMPROVEMENT OF OILSEED SUNFLOWER**

Marketability of oilseed sunflower has historically been dependent on the balance of fatty acids in the oil product, and the desirable balance of fatty acids has been subject to change over time. Among the drivers of change are consumer preference, based on clinical research, of reducing saturated fat in the diet, banning of trans-fats, and needs of food processors to have an oil with high oxidative stability. Sunflower oil products of the past were primarily composed of linoleic acid, which changed to 60-70 % oleic acid with the development of NuSun (mid-oleic) composition via mutagenesis, and is now shifting to high oleic compositions as high as 94 % oleic acid. With this shift, certain specialty markets set floors for oleic acid content of producer’s sunflower grain at delivery, which in certain environments has led to dockage because of unfavorable genotype-by-environment interactions. USDA-ARS has been working to better understand the nature of these interactions through multi-environment GWAS analysis, and has seen evidence of genes that are responsive to environmental differences. We are also studying the genetic architecture of very high oleic phenotypes, which appear to be the product of both major and minor quantitative trait loci. How this work feeds into existing sunflower breeding work will also be discussed.
STRUCTURE-FUNCTION RELATIONSHIPS IN RECOMBINANT PLANT DGAT1 VARIANTS WITH INCREASED PERFORMANCE

Guanqun (Gavin) Chen\textsuperscript{a}, Yang Xu \textsuperscript{a}, Rodrigo M. P. Siloto \textsuperscript{a}, Kristian Mark P. Caldo \textsuperscript{a}, Thomas Vanhercke \textsuperscript{b}, Anna El Tahchy \textsuperscript{b}, Nathalie Niesner \textsuperscript{b}, Yongyan Chen \textsuperscript{a}, Elzbieta Mietkiewska \textsuperscript{a} and Randall J. Weselake \textsuperscript{a}

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Diacylglycerol acyltransferase (DGAT) catalyzes the acyl-CoA-dependent acylation of sn-1, 2-diacylglycerol to produce triacylglycerol, the major component of seed oil. The level of DGAT1 activity can have a substantial effect on triacylglycerol production. Due to the lack of a three-dimensional detailed structure of DGAT1, structure/function insights into this membrane-bound enzyme remain limited. In this study, the amino acid residues governing \textit{Brassica napus} DGAT1 (BnaDGAT1) activity were investigated via directed evolution, targeted mutagenesis, \textit{in vitro} enzymatic assay, topological analysis, and transient expression of cDNA encoding selected enzyme variants in \textit{Nicotiana benthamiana}. The results revealed that numerous amino acid residues were associated with increased BnaDGAT1 activity. About 89\% of these sites were located outside the putative substrate binding or active sites. Moreover, 38\% of the identified sites are located near and within the 9\textsuperscript{th} transmembrane domain (TMD) on a predicted topology model. Two cDNAs encoding variants I447F or L441P within the 9\textsuperscript{th} TMD were transiently over-expressed in \textit{N. benthamiana} leaves. The leaves hosting variant L447F or L441P had 33.2\% or 70.5\% higher triacylglycerol content on a relative basis, respectively, compared to native BnaDGAT1. Overall, the results shed new light on structure-function in plant DGAT1 and the engineering of designer enzyme variants for increasing the oil content of plant biomass.
MODELING THE EFFECTS OF HIGH OLEIC OIL REPLACEMENT ON FATTY ACID INTAKE IN THE DIET OF US ADULTS.

Raatz S\textsuperscript{1}, Conrad Z\textsuperscript{1}, Jahns L\textsuperscript{1}, Belury M\textsuperscript{2}, Picklo M\textsuperscript{1}. \textsuperscript{1}USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND; \textsuperscript{2}Program of Human Nutrition, The Ohio State University, Columbus, OH.

Federally mandated removal of \textit{trans}-fatty acids from the US food supply by 2018 has introduced high oleic (HO) plant oils as replacements for the partially hydrogenated oils (PHO), the main source of \textit{trans}-fatty acids. This may result in substantial changes in the intake of fatty acids and may have widespread health implications. We aimed to predict the extent to which intake of fatty acids will change based on replacement of soy oil (SO) and canola oil (CO) with HOSO and HOCO in the diet. We therefore modeled the extent to which replacement of traditional SO and CO with HO varieties would affect the potential for meeting daily recommended intakes of individual fatty acids among US adults. Dietary and demographic data from 21,029 adults were acquired from the National Health and Nutrition Examination Survey (NHANES, 2007-2014), and the fatty acid profile of traditional oils and HO varieties were obtained from the USDA Nutrient Database and de novo analyses. We used several modeling scenarios: 10% replacement of current oils with HO varieties, 25% replacement, and 50% replacement. We demonstrated that each modeling scenario resulted in 1) minimally decreased intake of total SFA although intake remained higher than daily recommendations; 2) substantially increased intake of total MUFA, especially 18:1n-9; and 3) substantially decreased intake of total PUFA, especially 18:2n-6 and 18:3n-3. Increased use of HO plant oils in the food supply is predicted to have a major impact on fatty acid intake of the US adult population. The potential health effects of these changes are unknown.
DOCOSAHEXAENOIC ACID (DHA) ACTIVATION OF P38MAPK IN ENDOTHELIAL CELLS IS DEPENDENT ON GROWTH STATE

Youjia Du\textsuperscript{1,3}, Carla G. Taylor\textsuperscript{1,2,3}, Harold M. Aukema\textsuperscript{2,3}, Peter Zahradka\textsuperscript{1,2,3}

Department of Physiology and Pathophysiology\textsuperscript{1}, Department of Human Nutritional Science\textsuperscript{2}, University of Manitoba, and Canadian Centre for Agri-Food Research in Health and Medicine\textsuperscript{3}, St Boniface Albrechtsen Research Centre, Winnipeg, Manitoba, R2H 2A6

Introduction: Endothelial cells line the luminal surface of blood vessels and help maintain vascular homeostasis. DHA is thought to exert beneficial effects on vascular health by modulating endothelial function. However, a comparison of the effects of DHA on growing and confluent endothelial cells, which represent repairing and healthy states, respectively, is lacking.

Objective: To investigate the effects of DHA on viability and proliferation of subconfluent (growing) and confluent (quiescent) human EA.hy926 endothelial cells in relation to p38 mitogen-activated protein kinase (MAPK) activation.

Methods: Subconfluent or confluent EA.hy926 were treated with DHA (1-150 μM) for 24h followed by viability and proliferation assays. Phosphorylation of p38MAPK and mitogen-activated protein kinase kinase (MKK3/MKK6) was assessed after treating subconfluent and confluent cells with 125 μM DHA for 8h.

Results: DHA >125 μM reduced viability of growing and confluent cells after 24h, while 40 μM slowed proliferation of subconfluent and confluent cells. In subconfluent cells, 125 μM DHA activated p38MAPK and MKK3/MKK6 (a known upstream kinase), producing peaks at both 10 min and 4h, while those peaks were only observed at 10 min in confluent cells.

Conclusions: A dose-dependent effect of DHA on both proliferating and confluent endothelial cells with regards to viability and proliferation, and a growth state-dependent activation of p38MAPK were observed. Since activation of stress-stimulated protein p38MAPK, which is closely linked to pathological vascular remodeling, was observed in different growth conditions of cells, further studies to delineate the physiological effects of DHA on p38MAPK signal transduction in endothelial cells are warranted.
INTRANASAL SIMVASTATIN PREVENTS ALLERGEN CHALLENGE-INDUCED CHANGES IN LIPID METABOLITES

Christopher D. Pascoe, Aruni Jha, Thomas H Mahood, Sujata Basu, Michel Aliani, Andrew J. Halayko

Rationale: 10% of asthmatics exhibit refractoriness to currently used therapies. Simvastatin, which has pleotropic anti-inflammatory and anti-oxidant effects, has been shown in our lab to effectively resolve airway inflammation and hyperresponsiveness at low intranasal doses in a mouse model of asthma. We employed high throughput technology to understand how simvastatin modulates the lung lipid metabolome to resolve airway inflammation and hyperresponsiveness.

Hypothesis: Intransal simvastatin alters the lipid metabolome to promote pathways supporting lipid degradation.

Methods: Female BALB/c mice received intranasal HDM 5x per week for 2 weeks with or without concurrent SIN (6µg/kg/day). Bronchoalveolar lavage fluid (BALF) was collected 48 hours after the last HDM challenge. A non-targeted LC-QTOF-MS-based metabolomics methodology was used to detect lipid-derived compounds. Analysis of lipid signatures was done using multivariate methodology and online omics tools, MBrole 2.0 and Network Analyst.

Results: We identified 1480 metabolites in BALF which were significantly different between allergen-naïve, HDM, and HDM+SIN. HDM alone induced changes in 1095 unique metabolites (487 increased and 608 decreased) compared to naïve mice. Co-treatment with SIN and HDM prevented HDM-induced changes in 378 metabolites. First pass identity revealed that metabolites, altered by HDM and negated by SIN, exhibited associations to proteins responsible for phospholipid degradation, including: endothelial lipase, lipoprotein lipase, and phospholipase B1 (p<1x10^-10).

Conclusions: SIN prevents HDM-induced changes in metabolites associated with proteins responsible for phospholipid breakdown. Our new findings suggest that simvastatin delivered to the lungs may prevent the accumulation of bioactive oxidized lipids, in part by supporting their turnover.
Uncontrolled inflammation is a causative or perpetuating factor in many human disorders including obesity and associated metabolic diseases. There is evidence that dietary polyunsaturated fatty acids (PUFAs), including plant-derived \( \alpha \)-linolenic acid (ALA), can modulate inflammatory pathways. These lipids therefore provide an attractive therapeutic strategy, but a comprehensive understanding of their overall effects and specific modes of action is required. PUFAs can be converted to a variety of oxygenated metabolites known as oxylipins. Despite a relatively high abundance of ALA oxylipins in blood and tissues, very little is understood about their specific functions. Here, we investigate the direct effects of ALA oxylipins on an important innate immune cell type: the macrophage. First, we established cell culture models of resting (M0-like), activated (M1-like) and alternatively-activated (M2-like) macrophages. We then investigated how discrete ALA oxylipins modulate the phagocytosis and cytokine secretion functions of these macrophage populations. We found that acute exposure to the ALA oxylipin 12,13-DiHODE promotes phagocytosis by M2-like macrophages. Furthermore, exposure to certain ALA oxylipins during M1 or M2 polarization, including 9-HOTrE and 12,13-EpODE, reduces the secretion of pro-inflammatory cytokines such as TNF-\( \alpha \). In obesity, phagocytosis is generally protective while inflammatory cytokine secretion is pathogenic. Thus, these mediators appear to promote a beneficial macrophage phenotype. Through this study we hope to identify and describe novel, exploitable biological mechanisms by which dietary fats modulate immune function, inflammation and health.
ADULT RAT CARDIOMYOCYTE CELL DEATH INDUCED BY OXIDIZED PHOSPHATIDYLCHOLINES (OxPC) THROUGH THE FERROPTOTIC PATHWAY DURING ISCHEMIA/REPERFUSION

Aleksandra Stamenkovic¹, Kimberley A. O’Hara¹, David C. Nelson¹, Andrea Edel¹,², Grant N. Pierce¹, Amir Ravandi¹,²

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Background: Phospholipids represent the major constituent of biological membranes, with phosphatidylcholine (PC) being the most abundant one. Myocardial ischemia/reperfusion injury is followed by a large production of reactive oxygen species (ROS) leading to oxidation of PC and cardiomyocyte loss. The OxPC generated is associated with cardiomyocyte cell death. However, the mechanism of the cardiotoxic action of OxPC remains unknown. Ferroptosis, an iron dependent form of cell death is caused by accumulation of lipid oxidation products. Therefore, the main aim of our study was to examine the role of ferroptosis in OxPC-mediated cell death.

Methods: We utilized adult rat ventricular cardiomyocytes exposed to increasing concentrations of two different species of OxPC: PONPC(1-palmitoyl-2-(9'-oxo-nonanoyl)-sn-glycero-3-phosphocholine) and POVPC(1-palmitoyl-2-(5'-oxo-valeroyl)-sn-glycero-3-phosphocholine). Cell viability was measured by Live/Dead™ assay. Ferrostatin-1, an inhibitor of ferroptosis, was added together with an OxPC. To investigate the protective role of ferrostatin-1 in ischemia/reperfusion injury (I/R), cells were exposed to 1 h ischemia followed by 1 h of reperfusion, and ferrostatin-1 was added at the time of reperfusion.

Results: The two fragmented OxPCs, POVPC and PONPC, were cardiotoxic in a concentration dependent manner from 0.1µM-10µM. A 10µM concentration of POVPC significantly decreased cell viability (p<0.001). Treatment of cardiomyocytes with ferrostatin-1, added at the same time as OxPCs, attenuated POVPC-induced cell death (p<0.001) Treatment of cardiomyocytes with ferrostatin-1 also had a protective effect on cell survival during I/R.

Conclusion: These data demonstrate the cardiotoxic effects of OxPCs in cardiomyocytes and suggest a new therapeutic target and a therapeutic strategy for preventing cell loss in myocardial I/R injury.

Supported by grants from the Heart and Stroke Foundation
EARLY-LIFE EXPOSURE TO GESTATIONAL DIABETES ALTERS MITOCHONDRIAL BIOENERGETICS AND CARDIAC FUNCTION IN RAT OFFSPRING

Stephanie M. Kereliu1,2, Kyle G. Cheung1,2, Prasoon Agarwal1,2, Laura K. Cole1,2, Bo Xiang1,2, Mario A. Fonseca1,2, Grant M. Hatch1,2, Jonathan McGavock1,3, Vernon W. Dolinsky1,2

1Diabetes Research Envisioned and Accomplished in Manitoba (DREAM) Research Theme of the Children’s Hospital Research Institute of Manitoba, Departments of 2Pharmacology and Therapeutics and 3Pediatrics and Child Health, University of Manitoba. Winnipeg, MB, Canada

Introduction: Gestational diabetes mellitus (GDM) is the most common complication of pregnancy. Children of mothers with GDM are at increased risk for the development of cardiometabolic diseases later in life. We hypothesize that GDM induces fetal cardiomyocyte mitochondrial dysfunction, conditioning the offspring for the development of heart disease later in life.

Methods: To induce GDM, female rats were fed a high fat (45% kcal) and sucrose diet prior to mating, throughout pregnancy and lactation. Lean control females received a low fat (10% kcal) diet. Fetal rat ventricular cardiomyocytes (FRVCs) from e20.5 offspring were analyzed for mitochondrial respiration. Serial echocardiography was performed at e18.5 and at 3, 6, 9 and 12-months of age using a Vevo 2100 ultrasound. Metabolites from the serum of 3-month old offspring were measured using a UPLC-MS/MS interfaced with a HESI-II source and mass analyzer.

Results: Basal and maximal mitochondrial oxygen consumption was reduced for glucose (35% & 68%) and fatty acid (49% & 52%) substrates in FRVCs isolated from GDM offspring (p<0.05). Fetal and 3-month old offspring exposed to GDM exhibit increased left ventricle posterior wall thickness (p<0.05), a marker of cardiac hypertrophy. At 6- and 12-months of age offspring exposed to GDM exhibit increased isovolumetric relaxation time (p<0.05), indicating impaired diastolic heart function. Long chain fatty acids and acyl carnitines were elevated in the serum from 3-month old offspring, indicative of altered beta-oxidation.

Conclusion: GDM induced cardiomyocyte mitochondrial dysfunction in the offspring that was associated with the development of cardiac hypertrophy and diastolic dysfunction.
Plenary Speaker

William Harris, OmegaQuant Analytics, South Dakota

William Harris holds a PhD in Nutritional Biochemistry from the University of Minnesota. His research has focused on omega-3 fatty acids since 1980. He has been the principal investigator on 5 omega-3 related NIH grants. He has published over 200 papers on omega-3 fatty acids. In 2004, he co-developed with Clemens von Schacky, MD, the “Omega-3 Index”, a blood test to assess EPA+DHA biostatus. He is currently the President of OmegaQuant Analytics, LLC (Sioux Falls, SD) which offers the test to researchers, healthcare providers and consumers. He is also a Professor in the Department of Medicine at the Sanford School of Medicine, University of South Dakota.

OMEGA-6 AND OMEGA-3 FATTY ACIDS: PARTNERS IN PREVENTION

The purpose of this talk is to examine the effects of the two families of essential polyunsaturated fatty acids (PUFAs) - omega-6 and omega-3 – on risk for cardiovascular disease (CVD). Although most healthcare practitioners understand that the omega-3 fatty acids are beneficial, there has been considerable controversy about omega-6 fatty acids because of their presumed pro-inflammatory and pro-thrombotic effects. The American Heart Association’s Nutrition Committee has published three “Science Advisories” on these PUFAs: two on omega-3 (2002 and 2017) and one on omega-6 (2009). These advisories considered a wide variety of data from randomized trials, prospective observational studies, experimental studies, and animal and in vitro reports. The AHA concluded that Americans need to increase their intake of marine omega-3 fatty acids, and that they should maintain (or possibly even increase) their intakes of omega-6 fatty acids. Achieving healthy intakes of both omega-6 and omega-3 fatty acids is an important component of the nutritional prevention and treatment of CVD.
INCORPORATION OF DIETARY N-3 FATTY ACIDS INTO SELECTIVE PLASMA LIPIDS IN HUMANS AFTER SALMON INTAKE

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Elevated intake of n-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) is associated with reduced risk for cardiovascular disease. In this work, we tested the hypothesis that n-3 LCPUFA, namely docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are incorporated into select plasma phosphatidylcholine (PC) species and triacylglycerols (TAG) following intake of farm-raised, Atlantic salmon. Plasma samples were obtained from a randomized, cross-over designed study in which participants (\(n = 9\)) consumed salmon at 90 g and 180 g biweekly for 4 weeks. Plasma lipids were extracted and analyzed using a shotgun MS/MS method. Under basal conditions, DHA and EPA were incorporated into PC species containing palmitic acid (PA), stearic acid (SA), and oleic acid (OA) as the complementary fatty acid in the concentration order of PA>SA>OA. EPA content was enriched in PA, SA, and OA species following salmon intake whereas only PA and SA containing DHA species were elevated following intake. In contrast to PC, selective enrichment of DHA and EPA into TAG species containing an extra 1 or 2 desaturations and 34 carbons (consisting of PA and OA, palmitoleic acid and SA, or PA and linoleic acid) occurred. Few n-3 LCPUFA TAG species had two complementary saturated fatty acids. In conclusion, our data demonstrate that (1) n-3 LCPUFA incorporate into selective plasma PC and TAG pools some of which increase with dietary n-3 LCPUFA intake. These data allow for a more refined characterization of biomarkers for PUFA intake and understanding of LCPUFA biochemistry in humans.
Consumption of canola oil has been shown to lower blood cholesterol in individuals with hypercholesterolemia. However, little is known about how vascular function influences the response to LDL-cholesterol lowering. Our objective was to investigate the interactions among LDL-cholesterol, vascular function and parameters of the metabolic syndrome (MetS) in individuals consuming study foods providing 25 g/d of either traditional canola oil or a fat mixture representative of Western-type diets for 12 weeks. Participants had at least 2 characteristics of MetS and were not taking medications for blood lipids or glucose or body weight management. Assessments were conducted at baseline, 6 and 12 weeks. The Canola Oil Group had a significantly lower (P=0.0257) fasting total cholesterol at weeks 6 and 12 and a trend (P=0.0755) for a reduction in fasting LDL-cholesterol at week 12. Although blood pressure and blood vessel function were not altered over the 12 week study, canola oil consumption resulted in a greater reduction (-13% vs -1%) in LDL-cholesterol in individuals with healthier blood vessel function (small artery elasticity index [SAEI] >4.5%) compared to those with arterial stiffness (SAEI <4.5%). Those consuming the Western fat mixture and having healthier blood vessel function were resistant to the elevations in LDL-cholesterol (+6%) that were observed in those with arterial stiffness. This study has verified that dietary canola oil can lower blood cholesterol in individuals with MetS characteristics. These are the first data to implicate blood vessel elasticity as a factor that influences LDL-cholesterol responses to dietary fat type.
Plenary Speaker

Douglas Mashek, University of Minnesota

Dr. Mashek is Associate Director, Molecular and Cellular Basis of Obesity Core, Minnesota Obesity Center and Associate Professor, Departments of Biochemistry, Molecular Biology and Biophysics and Medicine, Division of Diabetes, Endocrinology and Metabolism, University of Minnesota-Twin Cities

Dr. Mashek’s research program is largely focused on fatty acid metabolism and lipid droplet biology and how they influence cell signaling and whole-body energy metabolism. His laboratory has developed broad expertise in metabolism ranging from whole-animal physiology to the molecular mechanisms regulating fatty acid trafficking and cell signaling. The current proposal builds upon builds upon his laboratory’s expertise in lipid droplets and opens up exciting research avenues that advance our understanding lipid metabolism and healthspan. Dr. Mashek is a member of the NIH-funded Minnesota Obesity Center and serves as an Associate Director of the Center’s Molecular Basis of Obesity Core, which provides support such as viral vector production, Seahorse energetics studies, animal physiology (energy expenditure, indirect calorimetry, etc.). He has a solid record of publications in the area of lipid droplet biology and metabolism in general and currently has multiple grants from the NIH and the American Diabetes Association.

BEYOND ENERGY STORAGE – THE LIPID DROPLET AS A SIGNALING NODE THAT REGULATES CELL FUNCTION AND METABOLISM

Lipid droplets are recognized to be the primary storage form of energy in most cell types. While this important function is critical to supply cells or organisms with energy during times of nutrient deprivation, their role beyond energy storage has largely been unexplored. Our laboratory has focused on elucidating the mechanisms through which lipid droplets communicate within cells to coordinate energy storage with cell signaling and metabolism. Our work on hepatic lipid droplets has identified important functions for these dynamic organelles in regulating lipid and glucose metabolism, hormone signaling and cell proliferation. Specifically, data will be presented showing that lipid droplet catabolism promotes lipid oxidation, gluconeogenesis and antagonizes the progression of the cell cycle. Moreover, recent insights into the mechanisms through which lipid droplets are catabolized will also be discussed. In summary, this presentation will highlight novel roles for lipid droplets and their metabolism in cellular and organismal biology and their importance in human health.
EXPRESSION OF HUMAN MONOLYSOCARDIOLIPIN ACYLTRANSFERASE-1 IMPROVES MITOCHONDRIAL FUNCTION IN BARTH SYNDROME LYMPHOBLASTS.

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The polyglycerophospholipid cardiolipin (CL) is remodeled to obtain specific fatty acyl chains in the mitochondrial membrane. This is accomplished by the transacylase enzyme tafazzin (TAZ) and monolysocardiolipin acyltransferase-1 (MLCL AT-1). Barth Syndrome (BTHS) patients with TAZ gene mutations exhibit impaired TAZ activity and loss in mitochondrial respiratory function. Previous studies have identified MLCL AT-1 as a mitochondrial enzyme capable of remodelling CL with linoleic acid. We examined if expression of MLCL AT-1 improves mitochondrial respiratory function in BTHS lymphoblasts. In healthy lymphoblasts reduction in TAZ expression resulted in a compensatory increase in MLCL AT-1 gene, protein and enzyme activity. BTHS lymphoblasts exhibited decreased TAZ gene and protein expression but in addition decreased MLCL AT-1 expression. Expression of MLCL AT-1 in BTHS cells resulted in increased CL, improved mitochondrial respiratory function and a decrease in the proportion of cells producing superoxide. Mitochondrial supercomplex (SC) assembly was significantly impaired in BTHS lymphoblasts and expression of MLCL AT-1 did not largely improve this phenotype. In addition, BTHS lymphoblasts exhibited higher rates of glycolysis compared to healthy controls probably to compensate for reduced mitochondrial respiratory function. The results suggest that expression of MLCL AT-1 is dependent on functional TAZ in healthy cells. In addition, expression of MLCL AT-1 may compensate, but not completely, for loss of mitochondrial respiratory function in BTHS lymphoblasts.
THE BLOOD BRAIN BARRIER AND FATTY ACID UPTAKE: ROLE OF THE BBB AS A METABOLIC BARRIER.

Eric J. Murphy

University of North Dakota, Biomedical Sciences, Grant Forks, USA

The blood brain barrier (BBB) functions as a physical barrier as well as a metabolic barrier in protecting the brain from the blood compartment. However, the role of the BBB in fatty acid uptake remains an enigma. Recent evidence suggests a multifaceted role for fatty acid transport proteins (FATP), fatty acid translocase (CD36), and fatty acid binding proteins (FABP) in facilitating fatty acid uptake. FATP are bifunctional proteins that facilitate fatty acid translocation while also forming an acyl-CoA in the process. On the other hand, CD36 is a well-known scavenger protein that has demonstrated fatty acid uptake in brain and other tissues. FABP are cytosolic proteins that act as molecular chaperones for fatty acids, with FABP5 found in the microvascular endothelial cells that form part of the BBB. While we have demonstrated that FABP3 is critical for brain arachidonic acid uptake, recent evidence by Hatch and colleagues demonstrates that arachidonic acid facilitates opening of the BBB through local formation of PGE$_2$ and interaction with the EP1 receptor. This novel pathway is different than previously known pathway associated with inflammatory response. While we are gaining an appreciation for the role of various proteins in fatty acid uptake and trafficking into the brain, the potential role for the BBB to form a fatty acid selective metabolic barrier is unappreciated. Previously, we demonstrated that [14-$^{14}$C]22:1n-9 (erucic acid) crosses the BBB and is taken up into the brain, but much less is taken up compared to arachidonic acid (about 20% as much). More importantly, this fatty acid which is rarely found in the brain, is rapidly chain shortened to 18:1n-9. However, when the tracer is infused directly into the fourth ventricle, most of the tracer is found intact as [14-$^{14}$C]22:1n-9 and is readily esterified. This suggests that the BBB may act as a metabolic barrier for fatty acids and metabolizes fatty acids into ones that are more readily found in the brain.
ALTERED HEPATIC FATTY ACID AND MITOCHONDRIAL METABOLISM CONTRIBUTES TO DEVELOPMENT OF GESTATIONAL DIABETES MELLITUS IN PREGNANT ADIPOSECTIN-DEFICIENT MICE

Brittany L. Moyce, Laura K. Cole, Bo Xiang, Mario A. Fonseca, Christine A. Doucette, Grant M. Hatch and Vernon W. Dolinsky

Introduction: Gestational diabetes mellitus (GDM) is characterized by hyperglycemia that develops during pregnancy. Clinically, there is an association between low levels of adiponectin and an increased risk for GDM. Adiponectin is a fat derived hormone that improves the sensitivity of tissues to insulin. We hypothesize that adiponectin deficiency causes fatty liver during pregnancy that impairs whole body glucose homeostasis.

Methods: We compared the glucose and insulin tolerance of pregnant (3rd trimester) adiponectin-/- and wild-type mice, and assessed mitochondrial function and fatty acid metabolism. Impact of adiponectin supplementation was determined by administering adenovirus mediated full-length adiponectin at the end of the second trimester of pregnancy, and comparing to control containing GFP.

Results: In the third trimester, pregnant adiponectin-/- mice exhibited fasting hyperglycemia (9.2mmol/L vs. 7.7mmol/L in controls, p<0.05) and impaired glucose and insulin tolerance relative to wild-type controls. Gestational weight gain and food consumption were similar in adiponectin-/- and wild-type mice. Hepatic triglycerides were elevated 3-fold in pregnant adiponectin-/- mice (p<0.05) due to a 2.5-fold (p<0.05) increase in fatty acid synthase expression and a 2-fold reduction (p<0.05) in maximal mitochondrial respiration when using fatty acids alone in hepatocytes isolated from pregnant adiponectin-/- mice. Adiponectin supplementation to pregnant adiponectin-/- mice improved glucose tolerance, prevented fasting hyperglycemia, and attenuated fatty liver development.

Conclusion: Adiponectin deficiency is associated with altered hepatic lipid metabolism and hepatic steatosis during pregnancy that contributes to insulin resistance and hyperglycemia characteristic of GDM. Adiponectin supplementation during pregnancy rescues insulin sensitivity and hepatic lipid metabolism in adiponectin-/- mice.
Dietary Oils and Sex Differentially Alter Rat Heart Oxylipin and Fatty Acid Profiles

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Oxylipins are bioactive lipid mediators produced from polyunsaturated fatty acids (PUFAs). The most well studied oxylipins are derived from arachidonic acid (AA, C20:4n6). Besides AA, other PUFAs such as linoleic acid (LA, C18:2n-6), alpha-linolenic acid (ALA, C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) exert many of their physiological effects via their respective oxylipins in different tissues, including the heart. For example, EPA derived 18-hydroxyeicosapentaenoic acid, protects against cardiac dysfunction, fibrosis and inflammation. However, the complete oxylipin profile in heart has not yet been reported. Dietary PUFA and sex can influence endogenous PUFA and oxylipin levels. Therefore, our objective was to profile heart oxylipins and to investigate the dietary and sex effects on the profiles. Growing male and female Sprague-Dawley rats were fed AIN93G based diets modified with oils having differing levels of ALA, EPA, DHA and LA for 6 weeks. Oxylipins and fatty acids were quantified from heart tissues by HPLC-MS/MS and GC, respectively. While in general oxylipins and their precursor PUFA profiles in heart were similar, notable exceptions were observed. For example, EPA derived heart oxylipins were higher in rats given ALA, EPA, and DHA diets while EPA itself was higher only in EPA diet. LA oxylipins, but not LA, were elevated in rats given the LA compared to the control diet. Unlike PUFAs, oxylipins were higher in female hearts. To conclude, heart contains a diverse array of oxylipins and there are diet and sex effects on these that do not necessarily reflect their precursor PUFA.
REGULATION OF HEPATIC INFLAMMATION BY FOLIC ACID IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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Non-alcoholic fatty liver disease (NAFLD) is a broad spectrum liver disorder that ranges from steatosis to steatohepatitis, cirrhosis and hepatocellular carcinoma. The histopathology of NAFLD is characterized by steatosis, inflammation, fibrosis, and liver injury. Chronic hepatic inflammation is an important feature of NAFLD that contributes to pathogenesis of the disease. There is currently no pharmacological agent approved for treatment of NAFLD. Dietary supplementation of vitamins is important for health maintenance, and has been suggested to be a beneficial strategy for NAFLD management. Folic acid is a water-soluble B vitamin that has been demonstrated to have lipid-lowering and anti-oxidant effects. The objective of our study was to investigate the effects of folic acid supplementation on hepatic inflammation and to identify the underlying mechanisms. Male C57Bl/6J mice were fed a control diet (10% kcal fat), a high-fat diet (60% kcal fat), or a high-fat diet supplemented with folic acid (26mg/kg diet) for a 5-8 week period. Folic acid supplementation reduced the aggregation of inflammatory foci as well as lipid accumulation in the liver induced by high-fat diet consumption. This correlated with reduced expression of pro-inflammatory cytokines mediated through inhibition of NF-κB transcription activity, an inflammatory transcriptional regulator. Folic acid supplementation did not alter the body weights of mice fed a high-fat diet. Our results suggest that folic acid supplementation can attenuate the hepatic inflammatory response induced by chronic consumption of a high-fat diet, which may contribute to the hepatoprotective effect by folic acid.

Funding source: This study was supported, in part, by Natural Sciences & Engineering Research Council of Canada (NSERC).

Key words: Folic acid, high-fat diet, inflammation
IDENTIFICATION OF BIOACTIVE LIPIDS DURING RENAL ISCHEMIA REPERFUSION (I/R) INJURY

Zahra Solati, Andrea Edel, Karmin O, Yue Shang and Amir Ravandi

Introduction: Ischemia/reperfusion (I/R) leads to series of cellular responses such as release of reactive oxygen species, elevation of apoptotic molecules, necrosis and release of bioactive compounds cause structural damage. Oxidized phosphatidylcholines (OxPCs) are the most abundant compounds produced during reperfusion due to oxidative stress. We have shown that bioactive OxPCs including 1-palmitoyl-2- (5'-oxo-valeroyl)-sn-glycero-3-phosphocholine (POVPC), 1-palmitoyl-2-glutaryl-sn-glycero-3- phosphocholine (PGPC) and 1-palmitoyl-2-(9'-oxo-nonanoyl)-sn-glycero-3-phosphocholine (PONPC) are involved in cell death pathways in myocardium during ischemia/reperfusion. The aim of this study was to identify these bioactive compounds in rat kidney during ischemia/reperfusion.

Methods: In rat kidney, ischemia was induced in eight male Sprague-Dawley rats (250-300 g) by clamping the left renal pedicle for 45 min followed by reperfusion for either 6h or 24h. As control, sham-operated rats were sacrificed at corresponding time points. Bioactive OxPCs were identified and quantitated using liquid chromatography coupled to electrospray ionization tandem mass spectrometry using an internal standard.

Result: Regarding rat kidney, following 6h of reperfusion, only PONPC in the I/R group was significantly elevated relative to the sham group (0.25±0.09 and 0.15±0.10 ng/mg, p<0.05). PONPC concentrations were even greater after 24h compared to 6h of I/R (p=0.014). However, after 24h reperfusion, POVPC, PGPC and PONPC were all significantly greater in the I/R group compared to the sham group (p<0.05).

Conclusion: We have shown for the first time that bioactive OxPC's increase during ischemia and reperfusion in Kidney model of I/R. Given that there are no therapies for renal I/R injury, inhibition of OxPC mediated renal injury can offer a novel avenue for therapy.
PRENYLTRANSFERASE EXPRESSION AND FUNCTION IN LUNG FIBROBLASTS FROM MODERATE COPD PATIENTS

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Chronic Obstructive Pulmonary Disease (COPD) is a chronic inflammatory disease that persists after cigarette smoking cessation, suggesting that lung fibroblasts are potential drivers of chronic bronchitis and emphysema. Fibroblasts from lungs of COPD subjects can exhibit a senescent, hyper-secretory phenotype which contributes to COPD pathogenesis. Prenyltransferases (PTs) facilitate membrane association and activation of key signaling proteins (Ex. Ras superfamily) through covalent conjugation of isoprenoid anchors. By inhibiting a specific PT (GGT-1), the secretory function of airway fibroblasts was previously shown to be modulated in asthmatic donors. We tested the hypothesis that PTs are responsive to cigarette smoke exposure (CSE) and are differentially regulated in lung fibroblasts from COPD and non-COPD donors. PT subunit labeling was evident in airway epithelium, airway smooth muscle and parenchymal fibroblasts in non-COPD lungs specimens. We observed no differences in PT subunit mRNA or protein abundance in cultured fibroblasts from COPD and non-COPD donors. CSE exposure increased GGT-1β protein abundance by 1.75 fold (t-test, p<0.01) in non-COPD lung fibroblasts. Using flow cytometry along with a fluorescent isoprenoid analog, exposure to CSE showed an increased PT activity regardless of patient status (p<0.05). In summary, CSE-induced GGT-1β accumulation occurs in cells from non-COPD donors however PT activity is increased by CSE exposure in COPD and non-COPD fibroblasts. These data suggest that PTs may be associated with regulatory mechanisms that determine patient-specific responses to environmental insult, and chronic disease pathogenesis.
A HIGH PROTEIN DIET ALTERS RAT KIDNEY AND LIVER OXYLIPINS

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The effects of high protein (HP) diets on tissues including the kidney and liver remain unclear. Oxylipins are formed via oxidative metabolism of polyunsaturated fatty acids via cyclooxygenase and lipoxygenase and cytochrome P450 enzymes. Some are involved in both the maintenance of normal renal and hepatic functions (prostaglandins), whereas epoxy-octadecadienoic acid (EpOME) and their dihydroxy metabolites (DiHOME) have been reported to be cytotoxic in renal cells and pro-inflammatory in a number of tissues. HP diets have been shown to alter renal prostaglandins, but effects on other oxylipins in the kidney or the liver are not known.

Normal adult male Sprague Dawley rats were provided isocaloric diets with LP (low protein, 8% protein by weight), NP (normal protein, 14%) or HP (50%) diets for two weeks. Kidney (cortex and medulla) and liver oxylipins were quantified by HPLC-MS/MS. Kidney and liver weights were higher in HP compared to NP and LP rats. Medullary DiHOMEs in HP rats were 2-3 times higher than in the NP and LP rats. EpOMEs followed the same trend. Several renal oxylipins from the cyclooxygenase and lipoxygenase pathways that may have protective effects were lower in the HP compared to the NP and/or LP diets. In liver, several oxylipins were higher in rats given HP compared to LP diets, including EpOMEs (2.5 times higher). In summary, HP diets result in oxylipin alterations in kidney and liver, including increased linoleic acid derived cytochrome P450 epoxygenase products. These findings warrant further investigation of their potential renal and hepatic effects.
THE EFFECT OF PRENATAL ALCOHOL EXPOSURE AND DOCOSAHEXAENOIC ACID SUPPLEMENTATION ON TESTICULAR DEVELOPMENT AND FUNCTION IN A RAT MODEL

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Although male fertility problems are diagnosed in adult life, evidence suggests that it might have a fetal origin. Both alcohol and dietary docosahexaenoic acid (DHA) are known to be testicular lipid modulators, which affect normal sperm and testosterone production, thereby affecting fertility. This study investigated whether dietary DHA and prenatal alcohol influence testicular development and function in a rat model. Forty pregnant Sprague-Dawley rats were randomly assigned to receive either ethanol (3g/kg, twice a day by gavage) or dextrose, isocaloric to ethanol, throughout pregnancy. Half of each group was fed either control or DHA supplemented diet (1.4%, w/w fatty acids). Respective diets were continued for the pups. Samples were collected at gestational day 20 (G20), postnatal day 4 (P4), P21, P49 and P90.

Dietary DHA significantly increased serum levels of testosterone at G20 and improved normal sperm morphology at P90. DHA diet showed a positive effect on testicular histological markers until puberty (P49). Compared to control groups, prenatal ethanol exposure affected the levels of testosterone in the serum from P4 to P90, with a delayed surge of testosterone. Prenatal alcohol induced a significant increase in expression of genes involved in testicular polyunsaturated fatty acids and seminolipid synthesis at P90. There were no significant changes in sperm motility and concentrations in both treatments at P90.

In conclusion, the results indicate that providing early dietary DHA can be a positive factor for male fertility with impacting on sperm morphology in adulthood. Prenatal alcohol exposure affects minimally sperm parameters in adult life.
PO-3

ATHEROGENIC DIET ENRICHED IN HIGH OLEIC CANOLA OIL AND OLIVE OIL Responds IN ANALOGOUS FATTY ACIDS AND LIPID PROFILE

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Numerous trials have shown that monounsaturated fatty acids (MUFA) improve lipid profile and reduce the cardiovascular disease (CVD) risk. High oleic canola oil (HOCO), olive oil (OO), and regular canola oil (RC) are rich in MUFA. However, in many countries, including Canada, HOCO is less expensive than OO and therefore, could be an attractive alternative which might carry similar effects on lipoprotein concentrations. Therefore, the overall objective of the study was to compare the metabolic effects of HOCO vs. OO in Golden Syrian hamsters. To address this, an animal trial (n=105) with seven different dietary oils was carried out for two months: (i) HOCO, (ii) OO, (iii) RC, (iv) blend of corn+safflower (C+S) (v) blend of flaxseed oil+safflower oil (F+S), (vi) HOCO+DHA (H+DHA), or (vii) HOCO+EPA (H+EPA). Plasma fatty acids were assessed by GC-FID. Plasma glucose and lipoprotein markers were analyzed using automated enzymatic methods on a Vitors-350 chemistry analyzer. Similar fatty acid profile, plasma glucose, and lipoprotein profile were observed between HOCO and OO treatments ($p<0.05$). No differences were found in total cholesterol (TC) among all dietary treatments ($p<0.185$). Furthermore, hamsters fed RC treatment decreased ($p<0.001$) non-HDL-C when compared with H+DHA. Data indicate that atherogenic diet differing in dietary fatty acid composition, when incorporated with either HOCO or OO responds in similar effects on plasma fatty acids, glucose levels, as well as lipoprotein profile. These factors will assist the consumer to make an informed decision that would best improve their lipid profile. (Supported by Natural Sciences and Engineering Research Council).
PO-4

DIETARY a-LINOLENIC ACID (ALA) IS SUFFICIENTLY CONVERTED TO DOCOSAHEXAENOIC ACID (DHA) TO INCREASE BIOACTIVE LIPIDS DERIVED FROM DHA

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Evidence for the beneficial effects of n-3 fatty acids on health has come primarily from fish oil feeding studies. However, plant oils can provide n-3 fatty acids in the form of α-linolenic acid (ALA), an 18-carbon fatty acid. Whether ALA can provide the beneficial effects of the longer-chain n-3 fatty acids such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) remains to be determined, but one argument against has been the apparent inability of ALA to be efficiently converted to DHA. Conclusions regarding ALA to DHA conversion are based on studies examining the effect of ALA on DHA levels. However, DHA mediates many of its effects via the production of its oxygenated bioactive lipid metabolites, called oxylipins (e.g. resolvins, protectins, hydroxy DHA). We have shown that dietary ALA can increase and restore low renal DHA oxylipin levels in models of pediatric renal disease without increasing the low DHA levels in diseased kidneys. Since tissue fatty acid levels are not always an accurate indication of oxylipin levels, the objective of this study was to determine whether dietary ALA can alter levels of DHA derived oxylipins in normal rat tissues. We show that even when dietary ALA does not alter tissue DHA levels, it can increase the levels of many oxylipins derived from DHA, indicating that the conversion from ALA to DHA can be sufficient to increase production of the biologically active DHA metabolites.
ALTERED ISLET FUNCTION MAY PROMOTE A LEAN PHENOTYPE IN TAFAZZIN DEFICIENT MICE

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Tafazzin is a transacylase that maintains mitochondrial membrane integrity and the function of the mitochondrial respiratory chain. Specifically, tafazzin maintains the content and molecular structure of the unique tetra-acyl phospholipid cardiolipin (CL) located in the inner mitochondrial membrane. Despite abundant evidence that mitochondrial dysfunction is associated with insulin resistance, little is known about the potential role of CL and tafazzin in the trajectory of this disease. To investigate the in vivo effects of tafazzin deficiency, we have utilized a mouse model with a doxycycline-inducible tafazzin shRNA knock-down. We have previously established that tafazzin knock-down mice are protected against the development of obesity, and insulin resistance compared to control litter mates. Tafazzin deficiency promotes a lean phenotype due to a coordinated elevation in adipose lipolysis and hepatic fatty acid oxidation. We have now determined that glucagon and insulin levels in the blood and whole islets are significantly reduced with tafazzin deficiency. We have also ascertained that the quantity and function of beta-cells were similar between genotypes. However, despite similar levels of alpha-cells, glucagon secretion during high-glucose conditions was elevated from islets isolated from tafazzin knock-down mice. As a result, tafazzin knock-down mice exhibited significantly higher circulating ratios of glucagon to insulin during glucose challenges. Our experiments indicate that tafazzin may have a role in regulating islet function. These data also suggest that mice deficient in tafazzin may be protected in part against weight gain by promoting glucagon secretion during fed states. Since, the development of type 2 diabetes is closely related to obesity and pancreatic function altering cardiolipin synthesis may be a novel therapeutic option for patients at risk for type 2 diabetes.
THE ANTI-DIABETIC AGENT BERBERINE INHIBITS MITOCHONDRIAL FUNCTION INDEPENDENT OF CARDIOLIPIN SYNTHESIS.

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Small clinical studies have shown that oral treatment with the plant alkaloid berberine reduces blood glucose levels similar to that of metformin and have promoted its use as a novel anti-diabetic therapy. *In vitro* studies have shown that high concentrations of berberine potently inhibit cell proliferation through inhibition of mitochondrial function. Cardiolipin is key phospholipid required for regulating mitochondrial bioenergetic function. We examined if berberine inhibited oxygen consumption rates in H9c2 cells through alteration in cardiolipin. Treatment of H9c2 cardiac myoblast cells with berberine resulted in a rapid (within minutes) concentration-dependent decrease in oxygen consumption rate. Concentrations as low as 1 µM rapidly inhibited oxygen consumption rate. Interestingly, treatment of H9c2 cells with up to 25 µM berberine for 24 h did not alter the pool size of cardiolipin nor its biosynthesis from fatty acid precursors. The results indicate that berberine treatment of H9c2 cells inhibits mitochondrial oxygen consumption rate independent of alteration in cardiolipin metabolism.
PHOSPHOKINOME ANALYSIS OF BARTH SYNDROME LYMPHOBLASTS IDENTIFY NOVEL TARGETS IN THE PATHOPHYSIOLOGY OF THE DISEASE

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Barth Syndrome (BTHS) is a rare X-linked genetic disease in which the specific biochemical deficit is a reduction in the mitochondrial phospholipid cardiolipin (CL). We compared the phosphokinome profile in Epstein-Barr virus transformed lymphoblasts prepared from a BTHS patient with that of an age-matched control patient. Mass spectrometry analysis revealed significant (>90%) reduction in CL mass and CL molecular species in BTHS cells compared to age-matched control cells. Phosphokinome analysis revealed striking differences in the phosphokinome profile of many proteins including proteins of which have previously been validated as bonafide phosphorylation targets in BTHS; For example, phosphorylated adenosine monophosphate kinase. Here we identify several novel phosphokinome targets in BTHS lymphoblasts and hypothesize that alteration in the phosphokinome profile may be involved in the pathophysiology of BTHS.
DOES RESVERATROL PROTECT AGAINST GESTATIONAL DIABETES AND THE RISK OF HEART DISEASE IN THE OFFSPRING?

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Introduction: Gestational diabetes mellitus (GDM), which affects 5-10% of pregnancies, is characterized by hyperglycemia in the third trimester of pregnancy. GDM increases cardio-metabolic disease risk in the offspring. Resveratrol (Resv), a naturally produced polyphenol, has anti-oxidant properties and positive metabolic health effects. We hypothesize that Resv administration (150 mg/kg) to pregnant GDM dams improves maternal glucose tolerance and protects offspring from GDM-induced obesity and heart disease.

Methods: Six weeks prior to mating, female rats consumed a high fat and sucrose (HFS) diet (45% kcal fat) to induce GDM, while lean control females received a low fat (LF) diet (10% kcal fat). In the third trimester, a subgroup of pregnant HFS-fed rats were supplemented with Resv (150 mg/kg). After weaning, offspring were randomly assigned a HFS or LF diet for 12 weeks. Offspring lipid levels were analyzed and echocardiography was used to assess cardiac function and morphometry.

Results: Resv improved maternal glucose tolerance, without affecting maternal body weight. Resv prevented GDM-induced elevated body and heart weights in the neonatal offspring (p<0.05). The body weights of GDM+Resv 15 week-old offspring were also lower than the GDM offspring (p<0.05). Liver and circulating triglycerides were reduced in GDM+Resv offspring versus GDM offspring (p<0.05). GDM+Resv offspring exhibited similar heart weights to that of lean offspring but had reduced left ventricular posterior wall thickness against GDM offspring (p<0.05). Functional parameters were unchanged in all groups.

Conclusion: Maternal Resv prevented obesity and cardiac hypertrophy in the offspring which are risk factors for heart disease.
Reducing the caloric and trans fat content of convenience foods has resulted in a significant growth in the market for food emulsifiers. Monoacylglycerols (MAGs), or mixtures with diacylglycerols (DAGs), are the focus of the food industry recently as these emulsifiers can be substituted to replace saturated and trans fats. Applying supercritical-CO2 (SC-CO2) as a low temperature technology for producing MAGs, investigation of the effect time and lipase on MAG yield and examination the efficacy of the phosphorous nuclear magnetic resonance (31P-NMR) technique for analysis the MAG-enriched oil composition, are the focus of this study. A SC-CO2 system was developed for the enzymatic alcoholysis of MAG-enriched oil from soybean oil in order to exclude the use of organic solvents. Production of MAGs, with and without lipase, were conducted under SC-CO2 condition at 70°C, 276 bar using 1,2-propandiol/soybean oil at a volume ratio of 3:4 by using Novozyme 435 lipase. MAG synthesis was greater under enzymatic conditions with the highest yield for the total MAG mix with DAG being 45% after 4 hours, compared to 1.2% for the non-enzymatic reaction. The ratio of the MAG to DAG was 1.2 after 4 hours in enzymatic reaction while this ratio for non-enzymatic reaction reached to the highest at 0.8 after 6 hours reaction. These findings contribute to the development of green approaches to value-added processing of soybean oil and address a critical industrial demand for solvent-free production of heat-sensitive emulsifiers at low temperatures.
Oxidized phosphatidylcholine induces COX2 gene expression and cytokine secretion by human airway smooth muscle cells

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Rationale: Mechanisms to maintain redox balance in the lungs are overwhelmed in severe asthma, resulting in oxidative stress that damages the phosphatidylcholine (PC)-rich lining of the lung forming oxidized 2-arachidonoyl-1-palmitoyl-sn-glycero-3-phosphocholine (OxPAPC). OxPAPCs are bioactive, with pro-inflammatory effects in numerous chronic diseases. In asthma, it is not known whether OxPAPCs promote a pro-inflammatory airway smooth muscle (ASM) phenotype.

Hypothesis: Oxidized phosphatidylcholine induce biogenesis of pro-inflammatory mediators through receptor mediated pathways in ASM cells.

Methods: Confluent cultures of primary human ASM cells from ex-smoker donors, were maintained in serum-free medium and treated (24hrs) with OxPAPC (0-160μg/mL) or PSPC, a fully saturated, non-oxidizable negative control. Lactate dehydrogenase (LDH) release was assayed to evaluate cytotoxicity and total mRNA and protein collected for qPCR and Western immunoblotting. Cytokine and oxylipin release in cell culture medium was also measured.

Results: OxPAPC caused a concentration-dependent moderate increase in LDH release. OxPAPC incubation also dose-dependently increased the abundance of mRNA and protein for COX2, but not prostaglandin D synthase or thromboxane synthase. OxPAPC exposure for 24 hrs also induced the release of IL-6, IL-8, and GM-CSF and 79 oxylipins including Prostaglandin B2. Western blotting data suggests that these effects are not through canonical TLR4 signaling mechanisms.

Conclusions: OxPAPC induces a pro-inflammatory phenotype in human ASM cells that is characterized by accumulation of mRNA for the prostaglandin synthesizing enzyme, COX2, concomitant with induction of cytokines, chemokines, and oxylipins that promote airway neutrophilia. Our data suggest that OxPAPC may underpin chronic airway inflammation in asthma.
OXIDIZED PHOSPHOTIDYLCHOLINE, A BIOACTIVE PRODUCT OF OXIDATIVE DAMAGE, CAUSES AIRWAY EPITHELIAL BARRIER IMPAIRMENT

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Rationale: Impaired epithelial barrier function plays a key role in the pathophysiology of asthma. Diminished tight junction integrity, including loss of E-cadherin and zonula occludens-1 (ZO-1), and decreased wound healing capacity increases the permeability of the epithelial layer to irritants. A phosphatidylcholine (PC)-rich lung lining fluid is susceptible to oxidative damage that results in peroxidation of unsaturated phospholipid chains, generating bioactive oxidized 2-arachidonoyl-1-palmitoyl-sn-glycero-3-phosphocholines (OxPAPCs). Dysregulated redox balance in the asthmatic lung increases the potential for excessive accumulation of OxPAPCs. We have shown that allergen challenge in mice and human asthmatics increases OxPAPC abundance in lung lavage fluid. Though OxPAPCs can promote inflammation and tissue damage, their effects in promoting epithelial barrier dysfunction are unknown.

Hypothesis: Oxidized phosphatidylcholine exposure causes the loss of tight junction proteins and impairment of airway epithelial barrier function.

Methods: The human airway epithelial cell line, Calu3, was grown to confluence, serum deprived and incubated with OxPAPCs or PSPC, a non-oxidizable negative control. After 24-hours cytotoxicity, cell stress, epithelial barrier function and permeability, wound repair, cellular ROS, and mitochondrial function was assessed.

Results: LDH release was evident only after 24hrs at the highest concentration of OxPAPC tested. Compared to PSPC, OxPAPC-treated cultures exhibited greatly reduced TEER, and increased trans-epithelial permeability. High concentrations of OxPAPC virtually abolished wound repair while lower concentrations delayed epithelium repair and barrier function restoration by ≥24 hours.

Conclusions: OxPC promote epithelial dysfunction by interfering with cellular redox and mitochondrial bioenergetics, which may contribute to asthma pathogenesis and symptoms.
PO-12

PEROXIDATION OF PHOSPHATIDYLCHOLINE: A SIGNATURE FOR ALLERGEN-INDUCED AIRWAY RESPONSES AND ALLERGEN SPECIFICITY

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Rationale: The late asthma response (LAR) characterizes severity of asthma exacerbations, however mechanisms are elusive. Allergen-challenge induced oxidative stress oxidizes phosphatidylcholine (PC) in the lungs to fragmented (f)/ un-fragmented (un-f) oxidized (Ox) PCs that drive cellular responses important for various disease pathologies. We hypothesize that in asthmatics, allergen induces unique changes in lung OxPCs that are associated to LAR-severity.

Methods: Segmental bronchial lavage was collected from 10 adult atopic mild asthmatics, 24 hours post- saline/ allergen challenge on separate days. LAR to saline/ allergen challenge was stratified as mild; moderate; and severe. Lipids were extracted and OxPCs were quantified from the lavage by LC-MS/MS. The correlation of OxPCs with LAR-severity and allergen was deciphered using supervised and unsupervised multivariate models.

Results: 13 unique OxPCs in human lung lavage detected had >90% overlap with OxPCs we catalogued in allergen-challenged mice. Total OxPC was similar after saline-(2.7±1.1ng/mL) or allergen-challenge (3.4±1.9 ng/mL). However, multivariate analysis distinguished the association of unique OxPCs with specific allergen and the corresponding LAR- saline triggered mild LAR and had a mixed signature of f- and un-f- OxPCs, while both house dust mite (HDM) and cat allergen induced severe LAR but were associated with different subsets of OxPCs. HDM was strongly associated with the accumulation of fragmented OxPCs while Cat-allergen was associated with un-fragmented OxPCs.

Conclusion: Our unbiased approach has revealed unique relationships between the allergen-induced severity and the abundance of specific bioactive OxPCs, suggesting OxPCs are important in asthma-pathophysiology, and may hold potential to predict for disease severity.
PO-13

CIGARETTE SMOKE EXPOSURE GENERATES A UNIQUE PEROXIDIZED PHOSPHATIDYLCHOLINE FINGERPRINT IN THE LUNG

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Rationale: Cigarette smoke (CS) induces oxidative stress and inflammation that underpins chronic lung disease pathogenesis. Under oxidative stress phosphatidylcholine (PC) rich lung surfactant and cell membranes undergo peroxidation (Ox) to form non-fragmented (nf-) and fragmented (f-) OxPCs that induces trophic and secretory responses in cells. CS likely induces OxPCs accumulation in the lungs, which has not been defined. Using lipidomics technology we profiled lung OxPCs and the effect of CS exposure in adult mice.

Methods: Female BALB/c mice (6-8 weeks) were exposed to room air (RA) or CS twice daily for 4 days using a smoke exposure chamber lung lavage were collected. Lipids were extracted from the lavage and OxPCs were subsequently identified and quantified by LC-MS/MS and analyzed using multivariate models.

Results: CS exposure induced inflammatory cell influx to the lung by 9-fold compared to RA (p<0.001). 23 OxPCs were detected in lung lavage, irrespective of exposure. Although CS-exposure did not change total OxPC abundance, CS-exposure generated a strong positive correlation with 10 nf-OxPCs. RA exposure was not correlated to any OxPCs observed. The nf-OxPCs that correlated with CS challenge included oxidized products of 1-palmityl-2-linoleyl-sn-glycero-3-phosphocholine (PLPC)-required for the synthesis of DPPC (chief component of surfactant).

Conclusion: CS exposure generates a unique OxPC fingerprint in the lung that is preferentially enriched in nf-PLPC subspecies, and not f-OxPCs as seen with allergen challenge. These changes suggest CS promotes oxidation of primary phospholipid components of surfactant and creates a unique microenvironment of bioactive OxPCs that could underpin CS-induced inflammation and tissue stress.
Spoilage or phytopathogenic fungi can affect the yields and nutritional quality of agricultural products; additionally, mycotoxins and fungal infections present significant food safety concerns. Some fungi have developed increased resistance toward common antifungal agents, increasing the amounts of fungicides required. Therefore, the development of alternative environmental-friendly antifungal agents is highly desirable.

Hydroxy unsaturated fatty acids (HUFA) are antifungal compounds found in active concentrations in fermented foods such as sourdough bread. Here, we investigated the production methods and in vitro antifungal activity of HUFA extracted from different matrices. Coriolic acid (13-hydroxy-9,11-octadecadienoic acid) was extracted from Coriaria seed oil, 10-hydroxy-12-octadecenoic acid from cultures of Lactobacillus hammesii and 13-hydroxy-9-octadecenoic acid from cultures of Lactobacillus plantarum TMW1.460Δlah, respectively. HUFA were purified by high-speed counter-current chromatography (HSCCC) and the fractions were characterized by LC/MS. Their antifungal activities were tested against filamentous fungi representing pathogenic and spoilage organisms, and yeasts that are relevant in food fermentations or spoilage. HUFA had a unique antifungal spectrum compared to other unsaturated fatty acids, as HUFA specifically inhibited filamentous fungi, including Aspergillus niger, Penicillium roqueforti, Aspergillus brasiliensis and Aspergillus clavatus with minimum inhibitory concentrations (g/L) of 0.29±0.07, 0.33±0.14, 0.50±0.25 and 0.25±0.00 respectively, while all yeasts were resistant to HUFA. Based on their inhibitory spectrum, HUFA were not effective against yeasts involved in food spoilage, however, they can be applied as antifungal agents in fermented foods that require growth and activity of yeast. Future experiments will be designed to explore the potential use of HUFA as food-grade antimold agents.
A leading animal model for asthma uses repeated intranasal (i.n.) challenge with house dust mite (HDM) in adult mice. Comprehensive analysis of the changes in the lung proteome with HDM challenge is sparse. The objective of this study was to use a proteomics approach to characterize and quantify global protein changes in the lung following HDM challenge. Female, BALB/c mice (6-8 weeks, n=3) were subjected to HDM i.n. challenge (25µg/mouse) 5 times per week for two weeks. Lung tissue from age-matched allergen-naïve and HDM challenged mice were homogenized prior to processing for HPLC-MS/MS. Proteins were identified and quantified (X!Tandem) prior to statistical (MEV) and pathway analysis (IPA). Of the 358 proteins that were significantly regulated by allergen challenge, 286 were up- and 72 down-regulated. Molecular networks preferentially activated by HDM challenge include Cdc42 and Rac signaling while down regulated pathways included Tight junction and Gβγ subunit signaling. Lipid metabolism and conversion pathways were predicted to be activated by allergen exposure. These lipid responses are driven in part by highly expressed oxidoreductases, a class of enzymes involved in mitochondrial and oxidative stress. Some of these proteins include Arachidonate 15-Lipooxygenase, Prostaglandin-Endoperoxide Synthase 1, and Fatty Acid Synthase. Using systems biology we have defined changes in the lung tissue proteome upon HDM challenge that results in altered lipid metabolism. Our platform provides an approach to decipher pathobiological mechanisms, identify new drug targets and assess response to therapy in chronic lung disease
SUSTAINABLE PRODUCTION OF BIODIESEL AND PROTEIN BY INTEGRATING ISOPROPYL ALCOHOL EXTRACTION AND RECOVERY FROM YELLOW MUSTARD

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Mustard is mass produced in Canada and can be used for both food and fuel markets without having adverse agricultural effects. While mustard hulls are useful food ingredients, mustard seed oil contains erucic acid which is responsible for its high lubricity, a positive property of bio-fuels. There is land availability to expand mustard production in Canada and in other arid areas to further increase its use for food and fuel production. Isopropyl alcohol (IPA) was used in developing a rapid method for oil extraction from yellow mustard to recover oil suitable for conversion to biodiesel. The recovery of IPA after oil extraction and biodiesel production was investigated to improve the efficiency and reduce the solvent cost. Multi-stage extraction improved oil recovery with up to 93.7% oil yield using 4-stage extraction at 2:1 IPA:flour (volume:weight) ratio at room temperature. IPA extracted mustard oil was converted to high quality esters with a 99% yield. As IPA forms a stable azeotrope with water improvements in IPA recovery were attempted by salting out IPA from the azeotrope with K$_2$CO$_3$. By using 20% K$_2$CO$_3$ (w/w of the mixture), 95% of the IPA was recovered at ~99% purity. Azeotropic distillation of the IPA-water azeotrope with 10% K$_2$CO$_3$ resulted in recovery of 99% of the IPA with 94% purity. The overall results suggest that IPA extraction followed by IPA recovery using salt enhanced azeotropic distillation is technically viable for near complete recovery of the oil and recycling of IPA from mustard flour extraction of oil and protein.
CHOLESTEROL AND PHYTOSTEROLS MODIFICATION IN REGULAR INFANT FORMULA IMPROVES CIRCULATING CHOLESTEROL LEVELS AND CHOLESTEROL SYNTHESIS IN A PIGLET MODEL.

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Several common diseases such as obesity, diabetes, CVD/CHD and other related health risk factors has been associated with high cholesterol synthesis. However, low cholesterol synthesis at infancy could subsequently lead to favourable cholesterol imprinting later in life. High synthesis at infancy could be traced to low dietary cholesterol especially in the formula-fed infants because they consume diet high in phytosterols (PS), a known cholesterol inhibitor, this high PS has been traced to vegetable oil used in supplementing infant food. Therefore, the aim of this study was to improve the circulating cholesterol level of an infant consuming formula by modifying dietary cholesterol and PS levels using piglets as model for human infants. A total of 32 piglets were used with 8 piglets per group in the following diet composition, high in PS; low in cholesterol (HpLc), high in PS; high in cholesterol (HpHc), low in PS; high in cholesterol (LpHc) and low in PS; low in cholesterol (LpLc). After 21 days of study, cholesterol and cholesterol synthesis levels in plasma and liver were compared across the four dietary treatment groups. In plasma and liver, cholesterol level was significantly higher in LpHc compared to LpLc. Synthesis levels in liver and plasma were also significantly lower in the LpHc diet group compared to HpLc and LpLc diet groups. In conclusion, lowering dietary PS may support healthy cholesterol levels while maintaining low cholesterol synthesis levels. These results are anticipated to help the manufacturing industries in proper formulation to achieve a closer dietary benefit found in human milk.
α-Synuclein (Snca) has an emerging role in brain arachidonic acid (20:4n-6) metabolism. Although Snca is important for microglial activation, its role in 20:4n-6 metabolism during neuroinflammation is unknown. Using Snca gene-ablated mice (KO), we determined the impact of Snca on brain 20:4n-6 metabolism during lipopolysaccharide-induced (LPS, i.p. 1mg/kg) inflammatory response in vivo using an established steady-state kinetic model. Radiolabeled [1-14C]20:4n-6 (170 mCi/kg) was infused (i.v.) 3h post-LPS injection into awake male mice. During the infusion, plasma was collected to generate a plasma curve and the unilateral incorporation coefficient determined for each metabolic compartment. Brain and liver [1-14C]20:4n-6 uptake and incorporation was measured in lipids separated using thin layer chromatography and the radioactivity determined using liquid scintillation counting. In liver, no significant differences were observed in [1-14C]20:4n-6 uptake or incorporation into lipid pools between groups. In Snca deficient mouse brain, there was a significant 1.3-fold increase in [1-14C]20:4n-6 uptake. This indicated more 20:4n-6 entering the Snca deficient brain from plasma. In the organic fraction, there was a significant 1.4-fold increase into total phospholipids in KO mice, accounted for by increased incorporation into choline glycerophospholipids and phosphatidylinositol. In neutral lipid pools, [1-14C]20:4n-6 incorporation into diacylglycerols (DAG) was reduced 75% in KO mice. Hence, under inflammatory conditions where 20:4n-6 release is enhanced, Snca has a crucial role in maintaining 20:4n-6 metabolism, and in the absence of Snca results in increased uptake and incorporation into lipid pools associated with enhanced lipid-mediated signaling during neuroinflammatory response.
COMPARISON OF DNA METHYLATION MARKERS IN FETAL RAT BRAINS PRENATALLY EXPOSED TO ALCOHOL TO HUMAN SUBJECTS WITH FETAL ALCOHOL SPECTRUM DISORDER

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The prevalence of fetal alcohol spectrum disorder (FASD) in Canada is closer to 1% and their excessive dependence on medical care puts huge burden on the health care system. DNA methylation associated with alcohol exposure could be a potential mechanism through which genes involved in neurogenesis are suppressed during fetal stages. The main objective of this study is to identify differentially methylated regions in rat fetal brains exposed to alcohol and compare it to methylation analyses of buccal epithelial cells from human subjects with FASD.

10 week old pregnant Sprague Dawley female rats received either ethanol 3g/kg body weight or dextrose twice a day by gavage. On G20 (gestational day 20), rats were terminated and brain tissues were harvested. Genomic DNA was isolated and DNA library was prepared by fragmentation, bead based enrichment of methylated fragments, adaptor ligation and amplification, emulsion PCR and sequencing on the SOLiD5500 platform. Data analysis showed that DNA methylation was significantly higher in alcohol groups, when compared to control group. Gene regions corresponding to methylation sites from rat brain samples were compared to genes identified to be hypermethylated in FASD subjects. The results shows that alcohol exposure increases DNA methylation in fetal rat brains. We have identified a number of genes methylated in both rat brain and human buccal epithelial cells. Many genes identified were associated to neuronal and cognitive function, and brain diseases. The current findings could be further explored for use as potential epigenetic marker for detecting FASD in newborns.
Adipose tissue is known to be a storage site for fatty acids in the body, but the extent to which it serves a metabolic role remains to be elucidated. Oxylipins are metabolites of PUFA, and are known to have metabolic properties which mediate the physiological effects of PUFA. In order to characterize the oxylipins in adipose and investigate how they are influenced by dietary fat, weanling rats were fed a control diet of adequate linoleic acid (LA) and alpha-linolenic acid (ALA), elevated LA, or elevated LA&ALA diets for 6 weeks. Mesenteric and gonadal adipose tissues were harvested upon termination to be analyzed for oxylipin content by solid phase extraction then LCMS analysis. The oxylipins in mesenteric and gonadal adipose were characterized and found to have different profiles; with 63 oxylipins in mesenteric and 51 in gonadal adipose. n-6 derived oxylipins were increased by the LA diet 19% and 11% in mesenteric and gonadal adipose respectively, with others increasing but not significantly. Additional dietary ALA diminished the increase to n-6 derived oxylipins. n-3 derived oxylipins were increased by the LA&ALA diet 4% and 22% in mesenteric and gonadal adipose respectively, with additional non-significant increases in gonadal adipose only. The LA diet did not alter any n-3 derived oxylipins. In conclusion, the LA diet increased both LA and arachidonic acid derived oxylipins, while additional ALA diminished this effect and increased ALA, EPA and DHA derived oxylipins.
THE EFFECTS OF DOCOSAHEXAENOIC ACID SUPPLEMENTATION ON GLOBAL GENE EXPRESSION IN RAT FETAL BRAIN WITH ETHANOL EXPOSURE DURING PREGNANCY

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Maternal nutrition status may protect the fetus from the external insults, such as alcohol. Prenatal alcohol exposure is known to affect genes involved in neurological development in fetal brains. Docosahexaenoic acid (DHA) is known to be a major membrane structural component of brain. Alcohol consumption is associated to decrease DHA levels in brain. This study investigated whether, DHA supplementation during pregnancy affects global gene expressions in fetal brain with chronic prenatal ethanol exposure. Pregnant Sprague Dawley rats were raised on one of the 3 experimental diets; control, DHA without ethanol, and DHA with ethanol. The diet was semi-purified, nutritionally rich and energy dense. Global gene expression in the fetal brains were analysed by microarray. qRT-PCR was used for validating microarray results. Protein expressions were analysed by western blot. No significant differences were identified in the fetal brains and body weights between the groups. Microarray analysis revealed that none of the transcripts were significantly altered in fetus brain. qRT-PCR, agreed with microarray data in fold changes. Ethanol significantly increased the expression of PCDHB6 whereas, DHA decreased it to the levels in control group. Ethanol also decreased WDR92 regardless of a DHA supplementation in comparison to the control. Western blot revealed that protein expressions were also not significantly different among the groups. In this study, moderate ethanol exposure or DHA supplementation during pregnancy have minor impact on rat fetal brain gene expressions. The nutrient dense diet provided in this study may have mitigated the effects of ethanol.