43rd Annual Canadian Lipoprotein Conference

Scientific Program

June 7th – 10th, 2018
Toronto Western Hospital
UHN-BMO Education and Conference Centre
About the 2018 Conference:

**Location of the Conference:**

Toronto Western Hospital,

BMO Education and Conference Centre

60 Leonard Ave, Toronto, ON, M5T 2R1

**Location of the Banquet:**

The Tall Ship Kajama

235 Queen’s Quay West,

Toronto, ON, M5J 2B8

**CLC Co-Chairs 2018**

Dr. Marica Bakovic, Professor, University of Guelph

Dr. Robin E. Duncan, Assistant Professor, University of Waterloo

**Contact Information:**

2018@lipoprotein.ca

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Thursday, June 7th
4:30-6:30 PM       Arrival and Registration
6:30-7:30 PM        Dinner
7:30-7:40 PM        Opening and welcoming remarks from Conference Chairs
7:40-8:10 PM        Stewart Whitman Young Investigator Award Lecture
                   Dr. Robin Duncan, Assistant Professor, Department of Kinesiology, University of Waterloo, Director of the Lipid Enzyme Discovery Lab
                   • A new enzyme & pathway in cardiolipin synthesis
                     SPONSORED BY AMGEN

Session I: Lipid Dysregulation – Diabetes & Cancer

8:10-8:40 PM        Dr. Gregory Steinberg, PhD, Professor and Tier II Canada Research Chair in Metabolism and Obesity, Division of Endocrinology, Department of Medicine, and Co-Director of the Metabolism and Childhood Obesity Research Program, McMaster University
                   • Cellular Energy Sensing and Metabolism

8:40-9:10 PM        Dr. Linda J. Z. Penn, PhD, Professor and Tier I Canada Research Chair in Molecular Oncology, Department of Medical Biophysics, University of Toronto, and Senior Scientist, Princess Margaret Cancer Centre, University Health Network
                   • Statins: a prescription for a long and healthy life

Friday, June 8th
7:30-8:30 AM        Breakfast (pastries, bagels, fruit, coffee/tea, juice)

Session II: Regulation of lipoprotein and cholesterol metabolism
(Chairs: Gordon Francis and Bernardo Trigatti)
8:30-8:45 AM        Dr. Yahya Ashraf (IRCM)
                   The Proprotein Convertase 7 Regulates Triglyceride Levels via Enhanced Apolipoprotein A5 Degradation
8:45-9:00 AM  Jacqueline Krysa (University of Alberta)  
ApoB-Remnant Dyslipidemia and High-Fat Meal Intolerance 
Exacerbates Cardiometabolic Risk in Overweight Children

9:00-9:15 AM  Joseph Longo (University of Toronto)  
Deregulated sterol metabolism is a targetable vulnerability of 
prostate cancer cells

9:15-9:30 AM  Dr. Stephen D. Lee (UCLA)  
IDOL regulates systemic energy balance through control of CNS 
VLDLR expression

9:30-9:45 AM  Dr. Elaheh Soleimannejad (University of Toronto)  
Lateral hypothalamic GLP-1 receptors regulate dietary fat 
absorption and intestinal chylomicron production

9:45-10:00 AM  Dr. Siavash Ghaaffari (University of Toronto)  
Physiological concentrations of estrogen decrease LDL transcytosis 
by coronary artery endothelial cells – elucidation of molecular 
mechanisms

10:00-10:15 AM  Refreshment Break

Session III: Treatment of Lipid Disorders 
(Chairs: Jessica Yue and Morgan Fullerton)

10:15-10:45 AM  Dr. Hoon-Ki Sung, MD, PhD, Scientist, The Hospital for Sick 
Children, Assistant Professor, Department of Laboratory Medicine 
and Pathology, University of Toronto  
- Intermittent Fasting Improves Metabolic Abnormalities in 
  Mice by Rejuvenation of White Adipose Tissue (Invited)

10:45-11:00 AM  Alex Rajna (University of Guelph)  
Alpha-linolenic acid and linoleic acid differentially regulate the 
skeletal muscle secretome of obese Zucker rats

11:00-11:15 AM  Paulina Aldana Hernandez (University of Alberta)  
Dietary choline supplementation does not increase atherosclerosis 
in atherogenic mouse models

11:15-11:30 AM  Emily A. Day (McMaster University)  
The SGLT2 inhibitor Canagliflozin activates AMPK and suppresses 
macrophage inflammation and liver lipogenesis in a mouse model of 
atherosclerosis
11:30-11:45 AM  Dr. Yosdel Soto (University of Alberta)
Novel anti-proteoglycan antibody inhibits subendothelial lipoprotein retention and prevents cardiac hypertrophy in a rat model of insulin resistance

11:45-12 PM  CLC Group Photo

12:00-1:30 PM  Lunch & Poster Session 1

1:30-2:30 PM  RUBENSTEIN LECTURESHIP
Dr. Edward A. Fisher, MD, MPH, PhD, Leon Charney Professor of Cardiovascular Medicine at the New York University (NYU) School of Medicine, Director of the Center for the Prevention of Cardiovascular Disease, and Director of the Marc and Ruti Bell Program in Vascular Biology
- Adventures in lipoproteins and atherosclerosis: yesterday, today, and tomorrow

Session IV: CIHR-INMD Joint Session: Dyslipidemia and treatment of insulin resistance (Chair: Gary Lewis)

2:30-3:15 PM  Dr. Gary Lewis, MD, FRCPC, UHN Senior Scientist, Toronto General Hospital Research Institute, Director, Banting and Best Diabetes Centre, Professor, Departments of Medicine and Physiology, University of Toronto
- Regulation of Lipid Mobilization and Chylomicron Secretion by the Intestine (Invited)

3:15-3:30 PM  Laís Perazza (Université de Laval)
Distinct roles of dietary fat and sugar in the development of obesity, insulin resistance, atherosclerosis and cardiac dysfunction in LDL receptor knockout mice

3:30-3:45 PM  Nadya M. Morrow (Western University)
Nobiletin prevents obesity, hepatic steatosis, dyslipidemia and insulin resistance independent of adipocyte AMP-activated protein kinase

3:45-4:00 PM  Mini Break
4:00-5:00 PM  **PHYSICIAN SCIENTIST LECTURESHIP**  
**Dr. Minna Woo**, MD, FRCPC, PhD, UHN Tier II Canada Research Chair in Signal Transduction in Diabetes Pathogenesis, Professor of Medicine and Medical Biophysics at the University of Toronto  
- Molecular signaling that interconnects metabolism and atherosclerosis  

5:00-6:30 PM  **Poster Session 2**  
- Reception (cash bar, light dinner - pizza, salad)

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**Saturday, June 9th**

7:30-8:30 AM  **Breakfast** (pastries, bagels, fruit, coffee/tea, juice)  

8:30-9:30 AM  **SIMON-PIERRE NOËL LECTURESHIP**  
**Dr. Barbara Karten**, PhD, Professor, Department of Biochemistry & Molecular Biology, Dalhousie University  
- Two aspects of cholesterol homeostasis: Mitochondrial cholesterol and cholesterol turnover in neurons

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**Session V**  
(Chairs: Katey Rayner and Hoon-Ki Sung)

**Part 1: Macrophage polarization and function**

9:30-9:45 AM  **Leah Susser (University of Ottawa)**  
Mitochondrial dynamics: A Tale of two Fs'

9:45-10:00 AM  **Dr. Peyman Ghorbani (University of Ottawa)**  
Choline transport links macrophage phospholipid metabolism and inflammation

10:00-10:15 AM  **Joshua A. Dubland (University of British Columbia)**  
Low expression of lysosomal acid lipase in arterial smooth muscle cells relative to macrophages provides insights into foam cell formation and a new therapeutic target for atherosclerosis

**Part 2: Genetics of obesity and atherosclerosis**  
(Chairs: Nica Borradaile David Mutch)

10:15-10:30 AM  **Jacqueline Dron (Western University)**  
Genetics of hypertriglyceridemia (HTG): an assortment of polygenic effects

10:30-10:45 AM  **Shannon L. Klingel (University of Guelph)**  
FADS1 genotype associated with human subcutaneous adipose tissue fatty acid profiles, but not inflammatory gene expression
10:45-11:00 AM  Michael A. Iacocca (Western University)
Whole-gene duplication of PCSK9 as a novel genetic mechanism for severe familial hypercholesterolemia

11:00-11:15 AM  Refreshment Break

11:15-11:45 AM  Dr. Yves Marcel Memorial and Celebration
Dr. Zemin Yao, Dr. Mireille Ouillmet, Dr. Scott Kiss

11:45 PM  Lunch (on own) & free time
Registered Trainees - Travel to Metro Toronto Convention Centre for YI Career Forum (starting at 12:15 PM)

12:10-1:10 PM  CLC Business Meeting (Salon C)

12:15-5:00 PM  Young Investigators Forum “Science: Career, Life and Beyond”
Hosted by CSATVB and CIHR (with ISA & CLC)
Registered trainees to depart at 11:45 AM for Metro Toronto Convention Centre

6:30-7:00 PM  Boarding the Tall Ship Kajama for Awards Banquet

7:00-11:00 PM  CLC 2018 Awards Banquet - Sunset/evening cruise of Toronto Harbour

11:00 PM  Return to dock

Sunday, June 10th
Departure and travel day
43\textsuperscript{rd} Annual
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2018 Lectureships
Dr. Fisher’s research program includes investigations of the cell biology of the very low density lipoproteins (the precursors of LDL), the regression of atherosclerosis, including its imaging, and the development of nanoparticles to target therapies directly to atherosclerotic plaques. He is also an active practitioner in preventive cardiology. He has published over 250 peer-reviewed articles on both clinical and research topics and has serving on many editorial boards, including the Journal of Clinical Investigation, Circulation Research, and Arteriosclerosis, Thrombosis, and Vascular Biology (of which he was the Editor in Chief). He has a number of honors, including: election to Alpha Omega Alpha (the national honor society of American medical schools) and the American Association of Physicians; the Solomon A. Berson Award in Basic Science Research Achievement (NYU); the Special Achievement and the George Lyman Duff Memorial Lecture Awards (American Heart Association) for contributions to atherosclerosis and lipid metabolism; and, the Pfizer/American College of Cardiology Visiting Professor of Preventive Cardiovascular Medicine, University of Virginia. From 2010-2011 he was the George Eastman Professor at Oxford University, an honor shared with 12 Nobel laureates. In 2016 Dr. Fisher received the National Lipid Association Achievement Award for “extraordinary expertise and contributions to the field of lipidology”.

Edward A. Fisher, MD, PhD
Edward A. Fisher, MD, PhD is the Leon Charney Professor of Cardiovascular Medicine at the New York University (NYU) School of Medicine. At NYU, he is also the Director of the Center for the Prevention of Cardiovascular Disease and Director of the Marc and Ruti Bell Program in Vascular Biology. He is a graduate of the NYU School of Medicine and received his clinical training at Duke and Harvard. He also holds a PhD from MIT in biochemistry and nutrition and was a post-doctoral fellow at the NIH in molecular genetics.

"Adventures in lipoproteins and atherosclerosis: yesterday, today, and tomorrow"
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Toronto, Ontario, Canada

Physician-Scientist Lectureship

“Molecular signaling that interconnects metabolism and atherosclerosis”

Minna Woo, MD, PhD

Dr. Minna Woo is a Professor in the Department of Medicine and Immunology at the University of Toronto. She is an endocrinologist and a clinician scientist, currently the Head of the Clinical Division of Endocrinology and Metabolism at the University Health Network/Mount Sinai Hospital and also the group leader for the Metabolism Division at the Toronto General Hospital Research Institute. She holds a number of distinctions in recognition for her research including the Canada Research Chair in Signal Transduction in Diabetes Pathogenesis. She was awarded the Young Scientist Award from both the CDA (now Diabetes Canada) and the Canadian Society of Endocrinology and Metabolism (CSEM), and is an elected member of the American Society for Clinical Investigation.
Simon-Pierre Noel Lectureship

“Two aspects of cholesterol homeostasis: Mitochondrial cholesterol and cholesterol turnover in neurons”

Barbara Karten, PhD

Dr. Karten obtained her undergraduate degree and MSc (Diploma) in chemistry at the University of Hamburg in Germany, before she moved to Graz, Austria for graduate studies. Her PhD research in the labs of Dr. Hermann Esterbauer and Dr. Wolfgang Sattler was mostly concerned with the analysis of cholesterol ester oxidation products during lipid peroxidation. As a postdoctoral fellow, Barbara joined the group of Dr. Jean Vance at the University of Alberta, Edmonton, where she got her first opportunity to work with primary neurons and Niemann-Pick Type C disease. At the end of 2005, Barbara joined the Department of Biochemistry and Molecular Biology at Dalhousie University in Halifax, where she is now a full professor. Her group’s research focuses on cholesterol metabolism in neurons and mitochondrial cholesterol homeostasis. In addition, she teaches an introductory biochemistry class, a graduate course on cell biology of lipids, and lectures in third and fourth year and medical school.
43\textsuperscript{rd} Annual
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ORAL PRESENTATIONS

SESSION I: Lipid Dysregulation - Diabetes & Cancer

Thursday, June 7\textsuperscript{th}, 2018
7:40-9:10 PM
43rd Annual Canadian Lipoprotein Conference
Toronto, Ontario, Canada

Stewart Whitman – CLC Young Investigator Award
(Sponsored by AMGEN)

“A new enzyme & pathway in cardiolipin synthesis”

Robin Duncan, PhD

Dr. Duncan is a Professor in the Department of Kinesiology at the University of Waterloo. She completed her BSc (Biological Sciences) at the University of Guelph, and PhD (Nutritional Sciences) at the University of Toronto (2004). From 2005-2010, Dr. Duncan completed a postdoctoral fellowship in the laboratory of Dr. Hei Sook Sul (Nutritional Sciences & Toxicology) at the University of California at Berkeley, where her work centred on the characterization of new enzymes in lipid metabolism. During this time, Dr. Duncan published studies in J Biol Chem, Nat Med, Diabetes, and Cell Metabolism on the molecular, cellular, and physiological functions of adipose triglyceride lipase, and AdPLA.

In 2011, Dr. Duncan joined the University of Waterloo, in the Physiology & Nutrition section of the Department of Kinesiology. There, she heads the Lipid Enzyme Discovery Lab that is focused on the identification, cloning and ‘molecules-to-physiology’ characterization of novel enzymes in phospholipid metabolism. Her work is funded by an Early Researcher Award (Ontario), and grants from NSERC, Diabetes Canada, CFI, the Waterloo Institute for Nanotechnology, and the Barth Syndrome Foundation. In addition, Dr. Duncan teaches 3rd and 4th year courses in micronutrient metabolism and nutrition, health and disease, and a graduate course in genetics.
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Toronto, Ontario, Canada

Invited Speaker
“Cellular Energy Sensing and Metabolism”

Gregory Steinberg, PhD

Dr. Steinberg is a professor of medicine at McMaster University where he holds a Canada Research Chair and a J. Bruce Duncan Endowed Chair in Metabolic Diseases. He is also a Co-Director of the Metabolism and Childhood Obesity (MAC) Research Program. His research studies cellular energy sensing mechanisms and how endocrine factors, lipid metabolism and insulin sensitivity are linked and contribute to the development of obesity, type 2 diabetes and cardiovascular disease.

Dr. Steinberg obtained his PhD in 2002 from the University of Guelph, where he studied the regulation of metabolism in muscle by the hormone leptin. From 2002-2006, Dr. Steinberg conducted Postdoctoral Research in the laboratory of Professor Bruce Kemp at St. Vincent’s Institute of Medical Research in Melbourne, Australia. During this time he gained insight into protein biochemistry and molecular biology with an emphasis on the metabolic stress sensing protein kinase AMPK. In 2006, Dr. Steinberg became Head of the Metabolism Unit at St. Vincent’s Institute of Medical Research and a Senior Fellow of the National Health and Medical Research Council of Australia. In 2009, Dr. Steinberg returned to Canada and joined the Department of Medicine, Endocrinology and Metabolism Division as an Associate Professor and Canada Research Chair. Dr. Steinberg has published more than 160 manuscripts, many in leading peer-reviewed journals (e.g. *Nature Medicine, Cell Metabolism, Diabetes*). Over the last year the impact of his work has been recognized by CIHR, Diabetes Canada, the Endocrine Society and the American Diabetes Association who have each presented him with Early Career and/or Outstanding Scientific Achievement Awards.
Linda Penn, PhD
Linda conducted her PhD at the University of Toronto and Hospital for Sick Children with Dr. Bryan Williams, focusing on interferon as an anti-viral agent. She then pursued her Post-Doctoral Fellowship at the Imperial Cancer Research Fund in London, UK with Drs. Hartmut Land and Gerard Evan, to study the MYC oncogene in cancer. Moving back to Canada, she established her lab with a focus on the regulation and function of MYC in cancer, and then added a second focus to the lab on evaluating the role of statin drugs as anti-cancer agents.

Dr. Penn is Senior Scientist at the Princess Margaret Cancer Centre and a Professor in the Medical Biophysics Department at the University of Toronto. She is also the inaugural Director of the University Health Network's Office of Research Trainees. She is a scientific advisor on several national/international review panels and is on several journal editorial boards. She has been honoured with several awards, the most recent being an Honorary Doctorate Degree from Linkoping University in Sweden.
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ABSTRACTS

ORAL PRESENTATIONS

SESSION II: Regulation of Lipoprotein & Cholesterol Metabolism

Friday, June 8th, 2018

8:30-10 AM
The Proprotein Convertase 7 Regulates Triglyceride Levels via Enhanced Apolipoprotein A5 Degradation by Yahya Ashraf |

Vatsal Sachan | Rachid Essalmani | Stephanie Duval | Jadwiga Marcinkiewcz | Josee Hamelin |
Anna Roubtsova | Annik Prat | Nabil G. Seidah | Laboratory of Biochemical Neuroendocrinology, IRCM |

In mice overexpression of type-I transmembrane human proprotein convertase 7 (hPC7), which cleaves secretory precursors after basic residues, leads to ~45% higher circulating triglycerides (TG) levels. In contrast, Afro-Americans expressing the hPC7 R504H variant had ~30% lower TG levels, suggesting that this substitution is a loss-of-function (LOF). Genome-wide association studies (GWAS) reported the association of 2 SNPs in the PC7 gene (PCSK7) with lipid parameters, one of which is in significant linkage disequilibrium with apolipoprotein A5 (APOA5).

The secreted ApoA5 activates lipoprotein lipase (LPL), and thereby accelerates the lipolysis of TG-rich lipoproteins, leading to TG storage in muscles and adipose tissue. In humans, ApoA5 LOF are associated with severe hypertriglyceridemia, whereas gain-of-function mutants are associated with increased uptake of TG-derived fatty acids by adipocytes.

To assess the link between PC7 and ApoA5 in vivo, we fed wild-type (WT) and PC7 knock-out (KO) mice a palm oil-rich diet and examined the effect of genotype on TG metabolism and ApoA5 levels. Like PCSK9 KO mice, PC7 KO mice also exhibited ~30% higher ApoA5 circulating levels.

Moreover, co-expression of PC7 and ApoA5 in hepatic cells led to reduced intra and extracellular ApoA5 levels, independently of the protease activity of PC7. Alkalinizing agents abolished this regulation, suggesting that ApoA5 degradation occurs in acidic compartments, likely endosomes/lysosomes. We further showed that PC7 phosphorylation at Ser505 is crucial for its activity on ApoA5, whereas ApoA5 degradation is dependent on its positively charged His-His183 doublet. Interestingly, PC7 directly interacts with ApoA5 close to its binding domain to LPL, consolidating PC7’s role in preventing LPL activation. Therefore, a PC7-inhibitor that prevents its interaction with ApoA5 may reduce plasma TG levels and possibly contribute to reduce hypertriglyceridemia.
ApoB-Remnant Dyslipidemia and High-Fat Meal Intolerance Exacerbates Cardiometabolic Risk in Overweight Children by Jacqueline Krysa | Donna Vine | Spencer Proctor | University of Alberta | University of Alberta

Cardiovascular disease (CVD) is proposed to develop in childhood and risk is exacerbated in obesity and the metabolic syndrome. However, the mechanisms explaining the etiologic link between early increases in adiposity and the pathogenesis of CVD in childhood remain unclear. Traditionally, fasting total-cholesterol and low-density lipoprotein cholesterol are used to identify children at risk of developing CVD, however these parameters do not reflect subclinical risk and CVD progression in children and levels are often normal in overweight/obese children.

Plasma apoB-remnant cholesterol is a lipid risk marker causally associated with CVD in adults. Fasting plasma apoB48 can be used as a marker of remnant cholesterol. High-fat meal intolerance (an accumulation of triglycerides and apoB48 following a high-fat meal) contributes to atherogenesis risk and is an independent subclinical predictor of CVD risk. In adults, fasting plasma apoB48 can strongly predict postprandial apoB48 following a high fat-meal challenge, demonstrating that elevated fasting apoB48 can be used as a biomarker of high-fat meal intolerance and CVD risk.

Our laboratory has provided some of the first data to suggest that obese pre-pubertal children (9.8 years; n=78) have elevated fasting apoB48-remnants compared to healthy-weight aged matched controls (23.5±1.1 versus 7.7±0.5 ug/mL). We have also demonstrated in a large cross-sectional study in children (9.6 years; n=570) that apoB48-remnants are highly correlated with central adiposity. Additionally, in a large prospective cohort (17.0 years; n=1045) we have shown apoB48-remnants are significantly elevated in adolescents with the metabolic syndrome (17.05±8.14 versus 13.24±5.41 ug/mL). Our preliminary data in lean children (n=12) also indicates fasting apoB48 is highly correlated with postprandial apoB48, similar to results in adults with apoB-remnant dyslipidemia.

Overall, our results demonstrate fasting and postprandial plasma apoB48-remnants are elevated in overweight-obese youth and high-fat meal intolerance may serve as an early
Deregulated sterol metabolism is a targetable vulnerability of prostate cancer cells by Joseph Longo | Peter J. Mullen | Rosemary Yu | Jenna E. van Leeuwen | Linda Z. Penn | Princess Margaret Cancer Centre & Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada | Princess Margaret Cancer Centre, Toronto, ON, Canada | Princess Margaret Cancer Centre & Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada | Princess Margaret Cancer Centre & Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada | Princess Margaret Cancer Centre & Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada

Statins are routinely prescribed for the management of hypercholesterolemia, but have also been shown to possess anti-cancer properties. We, and others, have shown that statins can induce cancer cell-specific apoptosis by inhibiting the mevalonate pathway, a crucial metabolic pathway responsible for the production of cholesterol and other non-sterol isoprenoids. In response to statin-mediated sterol depletion, cells initiate a homeostatic feedback mechanism governed by the sterol regulatory element-binding protein (SREBP) family of transcription factors. Recent data from our lab support that inhibition of this restorative feedback response in prostate cancer cells, as well as in other cancer cell types, potentiates statin-induced apoptosis. In line with these results, we recently identified dipyridamole, an agent approved for secondary stroke prevention, as an inhibitor of the SREBP proteins and a potentiator of statin-induced apoptosis, both in vitro and in vivo. Given that both statins and dipyridamole are approved for the prevention of cardiovascular disease, they are safe and immediately available to benefit cancer patient care.
IDOL regulates systemic energy balance through control of CNS VLDLR expression

by Stephen D. Lee | Christina Priest | Jie Gao | Prashant Rajbhandari |

Cynthia Hong | Peter Tontonoz | University of California, Los Angeles | University of California, Los Angeles | University of California, Los Angeles |

UCLA/Pfizer | University of California, Los Angeles

Liver X receptors limit the cellular uptake of lipids through transcription of the E3 ubiquitin ligase "Inducible Degrader of the LDL Receptor" (IDOL), which targets lipoprotein receptors for lysosomal degradation. We previously demonstrated that the LXR-IDOL pathway exerts species-specific effects on levels of lipoproteins through control of LDLR protein levels. However, the broader contributions of IDOL to systemic metabolism are unknown.

Here we show that loss of IDOL in mice is protective against the development of diet-induced obesity and metabolic dysfunction. Unexpectedly, analysis of diet induced obesity in a series of tissue-specific conditional knockout mice reveals that IDOL affects energy balance, not through its actions in metabolic tissues (Liver, Adipose, Endothelium, Intestine, Muscle), but rather through its actions in the central nervous system. We employed single-cell RNA sequencing of the hypothalamus to confirm these results, demonstrating that IDOL deletion altered key circuits that maintain central control of metabolism. Furthermore, by generating double knockout mice we identify VLDLR rather than LDLR as the key mediator of IDOL effects on systemic metabolism.

These studies identify a role for the IDOL-VLDLR pathway in the central nervous system in energy balance and susceptibility to diet-induced obesity.
Lateral hypothalamic GLP-1 receptors regulate dietary fat absorption and intestinal chylomicron production by Khosrow Adeli | Elaheh Soleimannejad | 1The Hospital for Sick Children & Depts Laboratory Medicine & Pathobiology, Biochemistry, and Physiology, University of Toronto | 1

Molecular Medicine Research Institute, The Hospital for Sick Children and Departments of Laboratory Medicine & Pathobiology, Biochemistry, and Physiology, University of Toronto, 555 University Ave, Toronto, ON, Canada

During the transition from fasting-to-fed states (immediately following meal ingestion), enteroendocrine cells in the proximal and distal small intestine are stimulated, possibly through a combination of neuro-hormonal pathways and direct nutrient stimulation, to secrete Glucagon-Like Pепides (GLP-1 and GLP-2) and several other gut peptides. Central control of intestinal lipoprotein metabolism via GLP-1 receptors was previously reported by our laboratory but the specific hypothalamic region(s) responsible for this regulation was unknown. We recently begun to directly map the GLP-1R containing neural pathways that control intestinal lipid metabolism. Among key neuronal populations, the lateral hypothalamus is one of the glucagon-like peptide-1 (GLP-1) receptor expressing brain regions which is innervated by the hindbrain GLP-1 neurons. GLP-1 is an incretin hormone regulating energy balance and chylomicron production centrally. We investigated the role of lateral hypothalamus-GLP-1 receptors in postprandial triglyceride-rich lipoprotein (TRL)-triglyceride production by bilateral microinjections of exendin-4 (GLP-1 receptor agonist) or exendin 9-39 (GLP-1 receptor antagonist), or both in Syrian golden hamsters. Intra- lateral hypothalamus injections of exendin-4 reduced TRL-triglyceride accumulation compared to vehicle group receiving PBS. This effect was prevented by bilateral injections of exendin9-39 into the lateral hypothalamus 15-20 minutes before exendin-4 administration. Bilateral microinjections of exendin9-39 alone had no effect on TRL-triglyceride production related to vehicle group. These results indicate the important role of lateral hypothalamus GLP-1 receptors in chylomicron production. Further studies need to distinguish molecular mechanisms involved in this process and elucidate the interplay between brain regions.

Key Words: Lateral hypothalamus, glucagon-like lipoprotein-1, chylomicron
Physiological concentrations of estrogen decrease LDL transcytosis by coronary artery endothelial cells - elucidation of molecular mechanisms by Siavash Ghaffari | Farnoosh Naderi Nabi | Warren Lee | St Micheal's Hospital | St Michael's Hospital | St Michael's Hospital

Abstract Id: 33
Submitted: May 18, 2018
Event: Canadian Lipoprotein Conference 2018
Topic: PDF award applicant

Objectives

The atheroprotective effects of estrogen are independent of circulating lipid levels and may be due to an effect on the vessel wall. Whether estrogen regulates transcytosis of LDL across the coronary endothelium is unknown.

Approach and Results

Using total internal reflection fluorescence microscopy (TIRF), we quantified transcytosis of LDL across primary human coronary artery endothelial cells treated with physiological concentrations of estrogen. Estrogen significantly attenuated LDL transcytosis by coronary artery endothelial cells from male donors; transcytosis of albumin was not affected. Estrogen caused down-regulation of endothelial SR-BI and over-expression of SR-BI by plasmid transfection was sufficient to restore LDL transcytosis. Similarly, depletion of SR-BI by siRNA attenuated endothelial LDL transcytosis and prevented any further effect of estrogen. In contrast, treatment with estrogen had no effect on SR-BI expression by liver cells from a male donor. Inhibition of estrogen receptors a and b had no effect on estrogen-mediated attenuation of LDL transcytosis. However, estrogen’s effect was blocked by depletion of the G-protein coupled estrogen receptor (GPER) by siRNA. GPER was found to be enriched in endothelial cells compared to hepatocytes and is known to signal via transactivation of the epidermal growth factor receptor (EGFR); we observed that inhibition of EGFR also prevented the effect of estrogen on LDL transcytosis. Lastly, male mice demonstrated more vascular deposition of fluorophore-tagged LDL than age-matched female mice after acute injection.

Conclusions

Physiological concentrations of estrogen significantly inhibit LDL transcytosis by down-regulating endothelial SR-BI; this effect requires GPER.
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ABSTRACTS

ORAL PRESENTATIONS

SESSION III: Treatment of Lipid Disorders

Friday, June 8th, 2018

10:15-11:45 AM
Hoon-Ki Sung, MD, PhD
Dr. Sung received his M.D. and Ph.D. from University of Yeungnam in South Korea. Following his Ph.D., he did basic research training at the Korea Advanced Institute of Science and Technology (KAIST) in the laboratory of Dr. Gou Young Koh. He moved to Toronto in 2006 and his postdoctoral training was in the laboratory of Dr. Andras Nagy in the Tanenbaum-Lunenfeld Research Institute at Mount Sinai Hospital. In 2014, he established his research laboratory in the Translational Medicine Program at the Hospital for Sick Children Research Institute. He is also cross-appointed to The Department of Laboratory Medicine and Pathobiology, University of Toronto. His main research interests include metabolism/adipose biology, angiogenesis and stem cell biology.
Intermittent Fasting Improves Metabolic Abnormalities in Mice by Rejuvenation of White Adipose Tissue by Hoon-Ki SUNG | Hospital for Sick Children

Ju Hee Lee¹², Eashita Das¹, Yun Hye Kim¹, Joanna Yeung¹, Yanqing Jiang¹, Min-Ah Choi³, Jae-Ryong Kim³, Hoon-Ki Sung¹²

¹Translational Medicine, The Hospital for Sick Children, Toronto, Canada
²Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada
³Department of Biochemistry and Molecular Biology & Smart-aging Convergence Research Center, Yeungnam University, Korea

White adipose tissue (WAT) undergoes dynamic remodelling in response to nutritional, environmental, and physical conditions. This process is essential for whole body energy homeostasis and metabolic adaptation of animals and human. Adipose tissue aging is a hallmark of age-associated metabolic dysfunctions. Age-induced impairment in adipose remodelling and adipose dysfunction lead to systemic inflammation and metabolic alterations, such as insulin resistance. In particular, recent studies demonstrate that adipose progenitor cells (APCs) adopt a fibrogenic phenotype to drive a pathological phenotype, indicating the importance of APCs in adipose tissue function. Previously, our study demonstrated that intermittent fasting (IF), a periodic energy restriction, protects mice from diet-induced obesity and associated metabolic abnormalities through white adipose tissue (WAT) browning and thermogenesis via VEGF-mediated adipose M2 macrophage polarization. As chronic inflammation/fibrosis is established in WAT by age and extended HFD, it is unknown whether IF will retain its beneficial effects in age-associated metabolic abnormalities.
Evidence shows that proteins secreted from skeletal muscle influence a broad range of metabolic signaling pathways. We previously reported that essential polyunsaturated fatty acids (PUFA) improved whole-body glucose homeostasis in obese Zucker rats; however, the mechanisms underlying these benefits remain enigmatic. While PUFA and obesity influence skeletal muscle function, their effects on the secretome are unknown. The aim of this work was to determine if improvements in whole-body glucose homeostasis in obese Zucker rats fed diets supplemented with either linoleic acid (LA) or alpha-linolenic acid (ALA) for 12-wks are related to changes in the skeletal muscle secretome. Secreted proteins were identified with a predictive bioinformatic analysis of microarray gene expression from red tibialis anterior (TA) skeletal muscle. Approximately 130 genes were differentially expressed (false discovery rate = 0.05) in obese rats compared to lean controls. The expression of 15 genes encoding secreted proteins was differentially regulated in obese controls, obese LA-supplemented and obese ALA-supplemented rats compared to lean controls. Five secreted proteins (Col3a1, Col15a1, Pdgfd, Lyz2, and Angptl4) were differentially regulated by LA and ALA. Most notably, ALA supplementation reduced Angptl4 gene expression compared to obese control and obese-LA supplemented rats, and reduced circulating ANGPTL4 serum concentrations. ALA also influenced Angptl4 gene expression and ANGPTL4 secretion from differentiated rat L6 myotubes. Altogether, the present data indicates that obesity has a greater global impact on skeletal muscle gene expression than either essential PUFA; however, LA and ALA may exert their metabolic benefits in part by regulating the skeletal muscle secretome.
Dietary choline supplementation does not increase atherosclerosis in atherogenic mouse models by Paulina Aldana Hernandez | Kelly-Ann Leonard | Nicole Coursen | Randal Nelson | Jonathan Curtis | Catherine Field | René Jacobs | 1Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton | 1 | 1 | 1Department of Biochemistry, University of Alberta, Edmonton | 1 | 1 | 1

**Background:** Choline, an essential nutrient, is required for cell membranes, lipoprotein secretion, and methyl-group metabolism. Recently it has been proposed that excess intake of choline is metabolized to trimethylamine (TMA) by gut microbiota; TMA is then oxidized to trimethylamine N-oxide (TMAO) by the liver enzyme, flavin-containing monoxygenase-3. It has been hypothesized that high TMAO levels in plasma may worsen cardiovascular disease. For example, choline-supplemented Apoe$^{-/-}$ mice show higher TMAO plasma levels and atherosclerotic size lesion compared to controls. The aim of this study was to investigate the dietary relationship between choline metabolites and atherosclerosis in two different atherogenic, Ldlr$^{-/-}$ and Apoe$^{-/-}$, mouse models.

**Methods:** A series of feeding trials were performed in Ldlr$^{-/-}$ and Apoe$^{-/-}$ male mice, aged 8-10 weeks. Mice randomly received control (0.1% choline), 5X choline- (0.5% choline), 10X choline- (1% choline), betaine- (0.1% choline and 0.9% betaine) or TMAO- (0.12% TMAO) supplemented diet, up to 28 weeks. After the dietary intervention, the animals were euthanized, and tissues and blood collected. Aortic atherosclerotic plaque area, plasma choline, and lipid metabolites were quantified.

**Results:** In Ldlr$^{-/-}$ mice, dietary supplementation with 10X choline or TMAO increased plasma TMAO levels by 1.6- and 4.1-fold, respectively after 8 weeks. Meanwhile, after 16 weeks there was an increase to 2-fold at TMAO supplementation. In Apoe$^{-/-}$ mice, plasma TMAO levels by 1.5-fold following 10X choline supplementation. Dietary betaine supplementation did not influence plasma TMAO levels. Despite the increase in plasma TMAO levels, dietary intervention did not alter atherosclerosis or plasma cholesterol levels in either mouse model.

**Conclusion:** In our study, high levels of dietary choline or TMAO supplementation did not influence atherosclerosis development. (Supported by CONACYT Mexico, NSERC, and ALMA).
The SGLT2 inhibitor Canagliflozin activates AMPK and suppresses macrophage inflammation and liver lipogenesis in a mouse model of atherosclerosis by Emily A. Day | Rebecca J. Ford | Jessie Lu | Rachel Lu | Eric M. Desjardins | Alex E. Green | James S.V. Lally | Jonathan D. Schertzer | Gregory R. Steinberg | McMaster University | McMaster University | McMaster University | McMaster University | McMaster University

Atherosclerotic cardiovascular disease (CVD) is the leading cause of death in the developed world and is characterized by chronic low-grade inflammation and dysregulated lipid metabolism. The AMP-activated protein kinase (AMPK) is a multifaceted protein kinase that increases fatty acid oxidation, suppresses de novo lipogenesis and exerts anti-inflammatory effects. Canagliflozin is a sodium-glucose transporter 2 inhibitor (SGLT2i) that is used to treat type 2 diabetes, but also exerts unexpectedly robust reductions in hospitalization and death due to CVD through unknown mechanisms. Recently, canagliflozin was shown to inhibit mitochondrial complex I leading to increased AMPK activity in hepatocytes, but whether this activation is important for mediating beneficial effects in a mouse model of CVD is not known. Canagliflozin treatment reduced adiposity, blood glucose, and liver lipogenesis, and increased fatty acid oxidation and energy expenditure in both ApoE\(^{-/-}\) and ApoE\(^{-/-}\)AMPK\(\beta_1\)^{-/-} mice. However, canagliflozin lowered serum IL-1b, TNFa and MCP-1 in ApoE\(^{-/-}\), but not ApoE\(^{-/-}\)AMPK\(\beta_1\)^{-/-} mice. Subsequent studies in macrophages found that canagliflozin, but not dapagliflozin or empagliflozin, reduced the phosphorylation of JNK and secretion of IL-1β through a pathway requiring AMPK, but not the NLRP3 inflammasome. These data indicate that canagliflozin reduces JNK-phosphorylation and IL-1β secretion from macrophages through a mechanism requiring AMPK and that this effect is associated with lower levels of circulating IL-1β in ApoE\(^{-/-}\) mice. Given the emerging role of IL-1β neutralizing agents for the treatment of CVD, these data suggest that canagliflozin may share a similar mechanism for improving CVD outcomes which requires further exploration.
Objective  Atherosclerosis is triggered by the arterial retention of ApoB-containing lipoproteins by proteoglycans (PGs). Previously, we characterized the antiatherogenic properties of a novel anti-PGs antibody (chP3R99) able to display an idiotypic vaccination effect. In this work we aimed at further assessing the impact of chP3R99 both on arterial lipoprotein retention and the cardiac function during insulin resistance. 

Methods  The blocking properties of chP3R99 antibody on lipoprotein retention was evaluated in solid-phase assays *in vitro* and *ex-vivo* by confocal studies in carotids from insulin resistant rats (JCR:LA-cp) either perfused with chP3R99 or immunized with this antibody for five weeks (200 ug, once a week). Cardiac function was assessed in immunized rats by echocardiography and western blot analysis. 

Results  chP3R99 antibody blocked over 50% the binding of both LDL and chylomicron remnants to vascular extracellular matrix from rats *in vitro*. Accordingly, pre-perfusion of carotids from JCR:LA-cp rats interfered with the arterial retention of LDL (~60%) and chylomicron remnants in a dose-dependent manner. The specific accumulation of chP3R99 antibody was verified in carotids from those rats, which also resulted in a significant reduction of arterial cholesterol deposition (*p*<0.05). Surprisingly, immunization with chP3R99 also avoided the development of cardiac hypertrophy in insulin resistant rats. This effect was characterized by a decreased expression of IL-4 and p-ERK 1/2 in heart tissue (*p*<0.05), along with increased levels of p-AKT as compared with the rats treated with the isotype-matched antibody. Immunization with chP3R99 induced an idiotypic antibody cascade in rat sera which contains anti-PGs antibodies whose accumulation could be detected both in the arteries and heart tissue. This findings suggest a protective role of those chP3R99-like antibodies both in lipoprotein retention and cardiac function. 

Conclusions  In conclusion, chP3R99 idiotypic antibody provides an innovative approach to target cardiovascular diseases.
43rd Annual
Canadian Lipoprotein Conference

ABSTRACTS

ORAL PRESENTATIONS

SESSION IV: CIHR-INMD JOINT SESSION

Dyslipidemia & Treatment of Insulin Resistance

Friday, June 8th, 2018

2:30-3:45 PM
43rd Annual
Canadian Lipoprotein Conference
Toronto, Ontario, Canada

CIHR-INMD Joint Session
Invited Speaker

“Regulation of Lipid Mobilization and Chylomicron Secretion by the Intestine”

Gary Lewis, MD
Dr. Gary Lewis completed his medical training in 1982 at the University of Witwatersrand in South Africa, followed by specialty training in Internal Medicine and then Endocrinology at the University of Chicago. He joined the staff of the Toronto General Hospital in 1990, was appointed Head of the Division of Endocrinology at University Health Network and Mount Sinai Hospitals in 2001, Director of the University of Toronto Division of Endocrinology and Metabolism in 2008 and Director of the Banting and Best Diabetes Centre, U of T, in 2011. He is a Full Professor in the Departments of Medicine and Physiology, University of Toronto and he holds the Sun Life Financial Chair in Diabetes and the Drucker Family Chair in Diabetes Research.

Dr. Lewis has made a number of important discoveries elucidating the mechanism of blood fat abnormalities in diabetes and prediabetic states. Dr. Lewis has been awarded and honoured by several national and international organizations. He has been invited to present his research findings at numerous universities around the world and at prestigious international meetings.
Distinct roles of dietary fat and sugar in the development of obesity, insulin resistance, atherosclerosis and cardiac dysfunction in LDL receptor knockout mice by Laís Perazza | Noemie Daniel | Marie-Julie Dubois | Patricia Mitchell | Khai Le Quang | Dominic Lachance | Thibault Varin | Rihab Bouchareb | Marjorie Boyer | Benoit Arsenault | Patrick Mathieu | Yves Pouliot | Sylvie Gauthier | Denis Roy | André Marette | (1) Quebec Heart and Lung Institute, Laval University, Quebec, Canada. (2) Institute of Nutrition and Functional Foods; Laval University; Quebec, Canada. | (1) Quebec Heart and Lung Institute, Laval University, Quebec, Canada. | (2) Institute of Nutrition and Functional Foods; Laval University; Quebec, Canada.

There is growing evidence that high intake of added sugar contributes to cardiovascular disease (CVD) but whether this is independent from high fat content in the diet remains to be established. To this end, our objective was to determine the impact of high sucrose intake on the features of metabolic syndrome and CVD in a mouse model of atherosclerosis.

Proatherogenic LRKOB100 mouse were fed either a low-fat/high-sucrose (LFHS) or a high-fat/low-sucrose (HFLS) diet for 24 weeks. Body weight gain, whole-body fat mass and insulin resistance were greater in HFLS vs. LFHS-fed mice. HFLS feeding also showed higher liver TG deposition and elevated plasma TG and cholesterol, while circulating HDL was lower compared to LFHS feeding. Although LFHS-fed mice were less prone to metabolic
impairments, they showed significantly more aortic plaque formation compared to HFLS-fed mice. Despite showing higher plasma HDL, LFHS-fed mice had lower cholesterol efflux capacity compared with HFLS-fed mice, which is consistent with the elevated lesion development in the aorta. 12-week echocardiography further revealed that LFHS-fed mice developed left ventricle eccentric hypertrophy vs. HFLS-fed mice. In addition, LFHS-related atherosclerosis and cardiac alterations were linked with higher hepatic levels the inflammatory cytokines IL-1β, IL-6, RANTES and TNF-α, vs. HFLS-fed mice. Our results indicate that high fat intake promotes obesity, insulin resistance and dyslipidemia compared to high sucrose intake. Conversely, intake of high sucrose lead to greater atherosclerotic lesion development and cardiac dysfunction which is linked to impaired HDL function and liver uptake of cholesterol esters, and hepatic inflammation.
Nobiletin prevents obesity, hepatic steatosis, dyslipidemia and insulin resistance independent of adipocyte AMP-activated protein kinase. by Nadya M. Morrow |
Andrew Y. Wang | Amy C. Burke | Dawn E. Telford | Brian G. Sutherland | Jane Y. Edwards | Murray W. Huff | 1Western University, Department of Biochemistry | 1 | 1 | 1 | 1 | 1 | Western University, Department of Biochemistry, Department of Medicine

In mice, diet-induced obesity contributes to insulin resistance, up-regulated hepatic de novo lipogenesis, adipocyte hypertrophy as well as the development of chronic adipose tissue inflammation. In Ldlr^-/- mice, supplementation of the citrus flavonoid nobiletin to a high-fat, high-cholesterol (HFHC) diet prevents obesity, insulin resistance, hepatic steatosis, and dyslipidemia, although the mechanisms are not fully understood. Studies in the literature show that nobiletin can induce the browning of cultured adipocytes, which was associated with activation of AMP-activated protein kinase (AMPK). Furthermore, recent studies in mice demonstrated that activation of adipocyte-specific AMPK stimulated white adipose tissue (WAT) browning, attenuated hepatic steatosis and improved glucose and insulin tolerance. Therefore, we hypothesized that induction of adipocyte AMPK was an integral part of nobiletin’s mechanism of action and that adipocyte AMPK deficiency would compromise metabolic protection mediated by nobiletin. Inducible adipocyte-specific AMPK knockout (iβ1β2AKO) male mice and their β1β2 floxed controls were fed HFHC (42% kcal fat, 0.2% cholesterol), or HFHC plus nobiletin (0.3% w/w) (n=8-10/group) for 12 weeks. The absence of adipocyte AMPK exacerbated the metabolic response to a HFHC diet. Unexpectedly, nobiletin supplementation robustly prevented obesity, hepatic lipid accumulation, dyslipidemia, insulin resistance, and improved energy expenditure in both iβ1β2AKO and control mice. Gross examination and histological analysis revealed that nobiletin decreased the mass of both inguinal (i) WAT, epididymal WAT, and reduced adipocyte size and number in both genotypes. Gene expression analyses demonstrated nobiletin did not affect the expression of browning genes, including Ucp1 and Serca2b, in iWAT in both iβ1β2AKO and control mice. In both genotypes, nobiletin equally protected against metabolic disorders, suggesting that the mechanistic action of nobiletin was independent of adipocyte AMPK. In conclusion, these studies further support that nobiletin prevents metabolic dysfunction in vivo, and that metabolic protection by nobiletin is conferred independently of adipocyte AMPK.
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ABSTRACTS

ORAL PRESENTATIONS

SESSION V:

Part 1: Macrophage Polarization and Function

Part 2: Genetics of Obesity and Atherosclerosis

Saturday, June 9th, 2018

9:30-11 AM
Mitochondrial dynamics: A Tale of two Fs'

by Leah Susser | Michele Geoffrion | My-Anh Nguyen | Katey Rayner | University of Ottawa Heart Institute | University of Ottawa Heart Institute | University of Ottawa Heart Institute

A hallmark of progressing atherosclerosis lesions is the recruitment of macrophages to the vessel wall and their subsequent polarization into pro-inflammatory (M1) macrophages, which sustain the inflammatory environment. In contrast, during lesion regression, macrophages adopt a pro-resolving, anti-inflammatory phenotype (M2). Each macrophage phenotype has distinct metabolic preferences in order to adapt to its tissue surrounds and functions, with M1 using glycolytic and M2 using oxidative metabolism to generate ATP. Mitochondria are highly dynamic organelles that are continuously undergoing cycles of fission and fusion, and have been shown in other cells to direct cell differentiation and function. We hypothesize that mitochondrial fusion and fission direct macrophage polarization towards an M1 phenotype drive atherosclerotic lesion progression. Analyzing mitochondrial length in M1 (LPS+IFNγ treated) and M2 (IL-4 treated) mouse bone-marrow derived macrophages (BMDMs), we observed an increase in fused elongated mitochondria in M1 macrophages compared to M2 or resting/control macrophages. Gene expression analysis showed that Drp1 a protein that directions mitochondrial fission, is increased in M1 vs. M2 macrophages whereas the expression of Mitofusins 1 & 2 were unchanged. We will now test whether Drp1, Opa1, Mfn1 & Mfn2 contribute to macrophage polarization by using BMDMs from mice with a genetic deletion of each member, followed by analysis of macrophage polarization and function. Because glycolytic metabolism by M1 macrophages creates a large number of reactive oxygen species (ROS) and ROS can cause damage to proteins, lipid membranes and mitochondrial structures, we will test the impact of ROS on the mitochondrial dynamics and the contribution to macrophage phenotype. These data will help understand how mitochondria, which are dynamic yet poorly understood organelles, may be contributing to inflammatory responses in the vessel wall during atherogenesis and other inflammatory diseases.
Choline transport links macrophage phospholipid metabolism and inflammation by Shayne A. Snider* | Kaitlyn D. Margison | Peyman Ghorbani | Nicholas D. LeBlond | Conor O’Dwyer | Thao Nguyen | Hongbin Xu | Steffany A. Bennett | Morgan D. Fullerton | University of Ottawa | University of Ottawa | University of Ottawa | University of Ottawa | University of Ottawa | University of Ottawa | University of Ottawa

Choline is an essential nutrient that is required for synthesis of the main eukaryote phospholipid, phosphatidylcholine (PC). Macrophages are innate immune cells that survey and respond to danger and damage signals. Although it is well known that energy metabolism can dictate macrophage function, little is known as to the importance of choline homeostasis in macrophage biology. We hypothesized that the uptake and metabolism of choline is important for macrophage inflammation. Polarization of primary bone marrow macrophages with LPS resulted in an increased rate of choline uptake and higher levels of PC synthesis. This was attributed to a substantial increase in the transcript and protein expression of the choline transporter-like protein-1 (CTL1) in polarized cells. We next sought to determine the importance of choline uptake and CTL1 for macrophage immune responsiveness. Chronic pharmacological or CTL1 antibody-mediated inhibition of choline uptake resulted in higher TNFα and IL-6 and lower IL-10 secretion in response to LPS. We further demonstrated that inhibition of choline uptake reduced the flux through the PC biosynthetic pathway and increased the levels of diacylglycerol (DAG) and activation of protein kinase C (PKC). Finally, to substantiate the link between the PC/DAG levels and inflammation, excess extracellular choline was fully and the PKC inhibitor bisindoylmaleimide was partially able to rescue aberrant cytokine secretion induced by choline uptake inhibition. These experiments establish a previously unappreciated link between choline phospholipid metabolism and macrophage immune responsiveness, highlighting a critical and regulatory role for macrophage choline uptake via the CTL1 transporter.
Low expression of lysosomal acid lipase in arterial smooth muscle cells relative to macrophages provides insights into foam cell formation and a new therapeutic target for atherosclerosis by Joshua A. Dubland | Sima Allahverdian | Ying Wang | Collin S. Pryma | Kamel Boukais | Michael A. Seidman | Teddy Chan | Gordon A. Francis

Background: Smooth muscle cells (SMCs) are the predominant cell type within the intima of human atherosclerosis-prone arteries, and promote the initial retention of atherogenic lipoproteins. Our recent evidence suggests SMCs comprise the majority of foam cells in human coronary atheromas, and that intimal SMCs have reduced ATP binding cassette transporter A1 (ABCA1) expression, previously shown to depend on the rate of release of cholesterol from lysosomes. **In the present studies we tested the hypothesis that SMCs have lysosomal dysfunction that contributes to foam cell formation.**

Methods and Results: Human monocyte-derived macrophages (HMMs) and arterial SMCs were treated with aggregated LDL (agLDL) to increase intracellular cholesterol. Unlike HMMs, agLDL treatment failed to upregulate ABCA1 expression in SMCs and did not
significantly increase 27-hydroxycholesterol levels. Also in contrast to HMMs, SMCs did not downregulate new cholesterol synthesis with agLDL loading. This data suggested retention of lipids within lysosomal compartments. Indeed, confocal microscopy revealed retention of lipids identified using the neutral lipid dye BODIPY within lysosomal compartments stained with LAMP1 in SMCs, while HMMs showed most lipids in cytosolic droplets. LIPA mRNA levels and LAL protein were markedly reduced in SMCs relative to HMMs, with LAL activity being 23.4-times higher in agLDL loaded HMMs when compared to SMCs (p<0.001). Interestingly, we found markedly decreased gene expression of LXRα and CYP27A1 in SMCs relative to macrophages. Despite these additional defects in sterol regulatory events, incubation of SMCs with medium containing LAL decreased lysosomal lipid accumulation leading to decreases in new cholesterol synthesis and increased cholesterol efflux.

**Conclusions:** Overall, we find that arterial SMCs have a relative deficiency in LAL and associated defects in downstream sterol regulatory events compared to macrophages. Our results indicate low LAL activity in SMCs as a novel reason for foam cell formation and a novel target to reduce atherosclerosis therapeutically.
Genetics of hypertriglyceridemia (HTG): an assortment of polygenic effects by Jacqueline Dron | Jian Wang | Henian Cao | Adam McIntyre | Irina Movsesyan | Mary Malloy | Clive Pullinger | John Kane | Robert Hegele | Robarts Research Institute, Western University | Robarts Research Institute | Robarts Research Institute | Robarts Research Institute | University of California San Francisco | University of California San Francisco | University of California San Francisco | University of California San Francisco | Robarts Research Institute, Western University

There are complex genetic determinants contributing towards hypertriglyceridemia (HTG), including rare and common variants in triglyceride (TG)-associated genes. Despite appreciating this spectrum of genetic influences, a comprehensive analysis in HTG patients for rare and common single-nucleotide variants (SNVs), and copy-number variation (CNVs) has not been reported. Here, we concurrently assessed rare SNVs, CNVs, and the accumulation of common SNVs in a 689 Caucasians with severe HTG (TG≥10 mmol/L). Each patient was sequenced using our targeted sequencing panel, LipidSeq. We first screened for rare SNVs and CNVs in TG-associated genes (LPL, APOA5, APOC2, GPIHBP1, and LMF1) and found 17.9% of patients carried rare variants with large phenotypic effects: 1.8% carried bi-allelic rare variants, while the remainder carried at least one heterozygous rare variant. We then assessed each patient for an accumulation of common SNVs using a polygenic risk score, and found 29.6% of patients had extremely high scores, indicating an excessive polygenic accumulation of common SNVs. Taken together, 47.2% of HTG patients had either a rare variant or polygenic burden likely contributing towards their HTG phenotype. After performing this analysis in a cohort of 503 healthy Caucasians from the 1000 Genomes Project, only 14.9% of individuals had either a rare variant or polygenic burden. Overall, HTG patients are >5-fold (CI 95% [3.81-6.76]; P<0.0001) more likely to carry a genetic factor linked to TG, compared to healthy individuals. Our study demonstrates the complex genetic determinants underlying severe HTG and provides insight towards the prevalence of each genetic factor. As sequencing technologies and bioinformatic analyses improve, the polygenic assortment of factors contributing towards HTG will be further characterized.
FADS1 genotype associated with human subcutaneous adipose tissue fatty acid profiles, but not inflammatory gene expression

by Shannon L. Klingel | Armand Valsesia | Arne Astrup | Marie Kunesova | Wim H.M. Saris | Dominique Langin | Nathalie Viguerie | David M. Mutch |
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Topic: Graduate award applicant

Background: Single nucleotide polymorphisms (SNPs) in FADS1/FADS2 genes are associated with changes in serum and tissue polyunsaturated fatty acids (PUFA) content. PUFA regulate inflammatory signalling pathways in adipose tissue; however, the effect of SNPs in FADS1/FADS2 on adipose tissue inflammation is equivocal. The present study examined if SNPs in FADS1/FADS2 modify human subcutaneous adipose tissue (SAT) fatty acid profiles and the expression of genes associated with inflammation/immune function, lipid metabolism and cellular differentiation.

Methods: SAT fatty acids and the expression of 117 genes were measured in 174 men and women from the DiOGenes Study using gas chromatography and qRT-PCR, respectively. Associations between fatty acids, gene expression, and SNPs in FADS1/FADS2 were investigated by linear regression and multivariate analysis.

Results: Four SNPs (rs174537, rs174546, rs174556, rs174601) in FADS1/FADS2 were significantly associated with SAT fatty acids. All SNPs were in high linkage disequilibrium with the commonly reported rs174537 SNP in FADS1. Minor allele carriers for rs174537 (GT+TT) had reduced 20:4n-6 (p=1.74E-5), lower delta-5 desaturase enzyme activity (p=2.09E-9), and lower FADS1 gene expression (p=0.03) compared to major GG carriers. Multivariate analysis revealed that 20:4n-6 and 20:3n-6 explained ~19% of the variance between rs174537 genotypes, while gene expression explained <7%. Receiver operating
characteristic (ROC) curves indicated that rs174537 genotype can be classified with SAT fatty acids (AUC=0.842), but not gene expression (AUC=0.627). No differences in SAT inflammatory gene expression were observed between rs174537 genotypes. SAT 20:3n-6 levels were positively correlated with the expression of several inflammatory genes, and inversely correlated with FADS1 expression.

Conclusion: This study showed that rs174537 in FADS1 is associated with SAT fatty acid profiles, but has little impact on adipose tissue inflammatory gene expression.
Whole-gene duplication of PCSK9 as a novel genetic mechanism for severe familial hypercholesterolemia by Michael A. Iacocca | Jian Wang | Thomas Lagace | Jacqueline S. Dron | Adam D. McIntyre | Paulina Lau | John F. Robinson | Ping Yang | Joan H. Knoll | Henian Cao | Ruth McPherson | Robert A. Hegele | Robarts Research Institute, Western University | Robarts Research Institute | University of Ottawa Heart Institute | Robarts Research Institute, Western University | Robarts Research Institute | University of Ottawa Heart Institute | Victoria Hospital, Western University | Victoria Hospital, Western University | Robarts Research Institute | University of Ottawa Heart Institute | Robarts Research Institute, Western University

Familial hypercholesterolemia (FH) is a heritable condition of highly elevated LDL cholesterol, characterized by premature atherosclerotic cardiovascular disease with increased risk of myocardial infarction and/or stroke. Here, we report two unique index cases who presented with severe FH: untreated LDL cholesterol of 14.9 mmol/L and 10.8 mmol/L, respectively, tendon xanthomata, extensive atherosclerosis culminating in cardiovascular events, and resistance to standard lipid-lowering therapies. Interestingly, targeted next-generation sequencing (NGS) of all FH major and minor/phenocopy genes LDLR, APOB, PCSK9, LDLRAP1, APOE, STAP1, LIPA, and ABCG5/8 by our LipidSeq panel showed no disease-causing mutations. In addition, copy number variation (CNV) analysis by multiplex ligation-dependent probe amplification in LDLR was also negative. However, with recent development of NGS bioinformatic tools CNV analysis can now be extended beyond LDLR, and retrospective CNV analysis of our LipidSeq data has revealed a whole-gene duplication of PCSK9 in both index cases; confirmed by microarray-based comparative genomic hybridization and whole-exome sequencing methods. This is the first report of a PCSK9 duplication discovered in FH. Given the established role of PCSK9 in promoting LDL receptor degradation, an increase in gene dosage leading to overproduction of PCSK9 represents a novel gain-of-function mechanism described for severely elevated LDL cholesterol. To further support this finding, we found this PCSK9 CNV to cosegregate with affected status in family members of both index cases. Furthermore, and perhaps most intriguing, plasma PCSK9 analysis performed in one index case revealed PCSK9 levels to be ~5000 ng/ml – a 21-fold increase compared to normal controls. Accordingly, this finding has therapeutic implications; these cases displayed a poor response statin therapies and require a high dose of a PCSK9 inhibitor for effective management. Lastly, this highlights the need to more routinely extend CNV assessment beyond the commonly screened LDLR, with
potential for finding novel disease-causing variants in additional FH-associated genes.
POSTER ABSTRACTS

Poster Session I
Friday, June 8th, 12:00-1:20PM

A2: The Regulation of PCSK9 by Lipoprotein Interactions
by Samantha Sarkar | Tanja Kosenko | Alex Foo | Natalie Goto | Thomas Lagace |
University of Ottawa Heart Institute | University of Ottawa

A4: The role of AMPK in the lipid disorder caused by Malaria infection in mice
by George Eduardo Gabriel Kluck | Camila Hübner Costabile Wendt | Maria Fernanda Carvalho Araújo |
Kildare Rocha Miranda | Georgia Correa Atella | Federal University of Rio de Janeiro

A7: The Natural History of Phytosterolemia: Observations on its Homeostasis
by David Mymin | Gerald Salen | Barbara Triggs-Raine | Darrel J Waggoner | Thomas Dembinski |
Grant M. Hatch | University of Manitoba | Rutgers New Jersey Medical School | University of Chicago

A8: The role of tafazzin and monolysocardiolipin acyltransferase-1 in mitochondrial function in Barth Syndrome lymphoblasts
by Edgard Mejia | Hana Zegallai | Eric Bouchard | Versha Banerji | Amir Rivandi | Grant M. Hatch |
University of Manitoba | Université de Saint-Boniface | St. Boniface Hospital Research Center

A9: Whole body knockdown of Mixed Lineage Kinase domain-like protein decreases circulating lipids but does not protect against atherosclerosis
by Adil Rasheed | Michele Geoffrion | MyAnh Nguyen | Richard Lee | Katey J. Rayner |
University of Ottawa Heart Institute | Ionis Pharmaceuticals

A12: Functional analysis of novel PCSK9 natural mutants from Sapiens (R96L, R105W and P174S) and Denisovan (H449L)
by Sepideh Mikaeeli | Emmanuelle Girard | Delia Susan Resiga | Josee Hamelin | Ali Ben Djoudi Ouadda |
Nabil G. Seidah | IRCM, Université de Montréal, Experimental Medicine, McGill University | IRCM, Université de Montréal

A17: Effects of acutely elevated mitochondrial cholesterol
by Luke Hattie | Barbara Karten | Dalhousie University, Department of Biochemistry and Molecular Biology

A18: Impaired hepatic phosphatidylcholine synthesis leads to cholestasis in mice
Sereana Wan | Folkert Kuipers | Rick Havinga | Dennis E. Vance | Rene L. Jacobs |
Jelske Van der Veen | University of Alberta | University Medical Center Groningen
A19: Large-scale deletions of the ABCA1 gene in patients with hypoalphalipoproteinemia
by Jacqueline Dron | Jian Wang | Amanda Berberich | Michael Iacocca | Henian Cao | Ping Yang | Joan Knoll | Karine Tremblay | Diane Brisson | Christian Netzer | Ioanna Gouni-Berthold | Daniel Gaudet | Robert Hegele | Robarts Research Institute, Western University | Western University | ECOGENE-21, University of Montreal | University of Cologne

A24: Effects of intervention with elongation factor 1A1 inhibitor, didemnin B, on NAFLD in western diet-induced obese mice
by Rachel Wilson, Cynthia Sawyez, Brian Sutherland, Richard Zhang, Taylor Woolnough, Nica Borradaile | Department of Physiology and Pharmacology, Western University

A25: Effects of low dose niacin and vitamin D on vascular regeneration under lipotoxic conditions
by Kia Peters | Zengxuan Nong | Chanho Park | Richard Zhang | Rachel Wilson, Hao Yin | Brian Sutherland | Cynthia Sawyez | J. Geoffrey Pickering | Nica Borradaile | Department of Physiology and Pharmacology, Western University | Robarts Research Institute | Western University

A26: Examination of the relative roles for oxysterol-binding protein homologues mediated non-vesicular and vesicular transport pathways in the subcellular distribution of phosphatidylserine
by Yanbo Yang | Gregory D. Fairn | Keenan Research Centre for Biomedical Science, St. Michael's Hospital | Department of Biochemistry, University of Toronto | Department of Surgery, University of Toronto

A27: Elevated Mitochondrial Cholesterol and its Effects on the formation of ER-Mitochondria Contact Sites
by Aaron Woblistin | Barbara Karten | Dalhousie University

A29: Molecular mechanisms of feedback inhibition of sphingolipid biosynthesis by Orm proteins
by Omar Mourad | Gregory D. Fairn | University of Toronto | St. Michael's Hospital

A30: 7-Ketocholesterol Impairs Phagocytosis and Efferocytosis via Dysregulation of Phosphatidylinositol 4,5-bisphosphate
by Stella Lu | Department of Biochemistry, University of Toronto | Keenan Research Centre for Biomedical Sciences, St. Michael's Hospital
A34: Arresting progression and promoting regression of atherosclerotic lesions: a dose-dependent effect of active immunization with an antiglycosaminoglycan monoclonal antibody
by Victor Brito | Katia Mellal | Roger Sarduy | Karina F. Zoccal | Yosdel Soto | Liliane Ménard | Lucia H. Faccioli | Huy Ong | Ana Maria Vázquez | Sylvie Marleau | Faculté de pharmacie, Université de Montréal | Division of Immunobiology, Center of Molecular Immunology, Havana, Cuba | Department of Clinical Analysis, Toxicology and Bromatology, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo | Innovation Managing Direction, Center of Molecular Immunology, Havana, Cuba

A37: Postprandial Lipid Dynamics and Intestinal GLP-1 Response in Healthy and Obese Adolescents
by Victoria Higgins | Man Khun Chan | Khosrow Adeli | The Hospital for Sick Children, University of Toronto

A39: miR-130b modulates hepatic lipid metabolism and potently upregulates VLDL Production
by Jing Zhang | Danielle Alvares | Ferdous Rastgar Jazii | Negar Khrosraviani | Lipei Liu | Khosrow Adeli | Hospital for Sick Children, University of Toronto

A40: Validation of the AMPK-SREBP2 pathway and implications on hepatic cholesterol homeostasis
by Julia R.C. Nunes | Peyman Ghorbani | Morgan D. Fullerton | University of Ottawa Center for Infection, Immunity and Inflammation | Ottawa Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa

A41: Hepatic cholesterol dysregulation
by Ting Xiong | Pei Yu | Paul Lebeau | Richard C. Austin | Bernardo L. Trigatti | McMaster University

A42: Phosphoethanolamine reverses characteristics of metabolic syndrome in Pcyt2(+/-) mice
by Gabriela Mattos | Joy Oresajo | Dr. Marica Bakovic | University of Guelph

A43: Scavenger receptor class B type 1 knockout mice develop extensive dietinduced aortic sinus and coronary artery atherosclerosis in an age-dependent manner
by Samuel K Lee | Mark T Fuller | Bernardo L Trigatti | McMaster University

A57: The lysophosphatidic acid acyltransferases (acylglycerophosphate acyltransferases) family: one reaction, five enzymes, many roles
by Ryan Bradley | Ashkan Hashemi | Emily Mardian | Juan Jose Aristizabal Henao | Darin Bloemberg | Joe Quadrilatero | Ken D. Stark | Robin E. Duncan | University of Waterloo
A44: Thermogenesis independent metabolic benefits conferred by intermittent fasting in ob/ob mice
by Yun Hye Kim | Ju Hee Lee | Kyoung-Han Kim | The Hospital for Sick Children | University of Ottawa Heart Institute

A45: Mechanism and efficacy of statins as metastasis-prevention agents in breast cancer
by Jenna E van Leeuwen | Rosemary Yu | Joseph Longo | Cunjie Zhang | Wenjiang Zhang | David Cescon | Eric Chen | Richard R Drake | James W Dennis | Linda Z Penn | Princess Margaret Cancer Centre, Toronto, ON, Canada | Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada | Medical University of South Carolina

A46: Free Fatty Acids Liberated by Lipoprotein Lipase from Lipoproteins Inhibit Cholesterol Efflux by Activating Akt
by Jenika D. Marshall | Bronwyn A. Woolfrey | Emily R. Courage | Madeline N. Fitzpatrick | Ryan F. Elliott | Yanbo Yang | Robert J. Brown | Memorial University of Newfoundland

A47: Ethanolamine Transport and the Choline Transporter-like Protein 1 (CTL1)
by Adrian Taylor | Marica Bakovic | University of Guelph

A48: High Density Lipoprotein Mediated Protection of Macrophages against Apoptosis Requires Scavenger Receptor Class B Type 1 Activity and Sphingosine-1-Phosphate
by Kevin Chathely | Bernardo Trigatti | McMaster University

A49: PCSK9 Mediated Degradation of the LDL Receptor is Influenced by Ca^{2+} Levels in the Early Endosome
by Curtis Brandt | Thomas A. Lagace | University of Ottawa Heart Institute

A50: Regulation of Lipid Homeostasis by Glucocorticoids in the Mediobasal Hypothalamus
by Emilie Beaulieu-Bayne | Jennifer Lee | Jessica Yue | Department of Physiology, University of Alberta

A51: Studies of effects of LysMcre/creS1P1lox/lox mutations on S1P1 expression in different tissues of atherogenic mice
by Narmadaa Thyagarajan | Leticia Gonzalez | Darren Sam | Usama Tahir | Bernardo Trigatti | McMaster University
A52: Portal vein GLP-1 receptor activation modulates dietary fat absorption and intestinal lipoprotein metabolism
by Simon Hoffman | Khosrow Adeli | Molecular Medicine, Research Institute, The Hospital for Sick Children and University of Toronto

A53: The Scavenger Receptor Class B Type I regulates the levels of the Sphingosine 1- Phosphate Receptor 1 in mouse peritoneal macrophages
by Christine Bassila | Bernardo Trigatti | McMaster University

A54: Depletion of lesion macrophages characterizes atherosclerosis regression induced by naringenin
by Amy C. Burke | Brian G. Sutherland | Dawn E. Telford | Marisa R. Morrow | Cynthia G. Sawyez | Jane Y. Edwards | Murray W. Huff | University of Western Ontario

A55: Effects of hematopoietic deficiency of pro-apoptotic protein Bim on the development of atherosclerosis
by Alexander Qian | Pei Yu | Leticia Gonzalez | Bernardo Trigatti | McMaster University

A56: Statins cause IL-1β-dependant adipose insulin resistance via lower protein prenylation
by Brandyn Henriksbo | Joshua Xu | Jobanjit Phulka | Britanny Duggan | Joe Cavallari | Kevin Foley | Akhilesh Tamrakar | Jonathan Schertzer | McMaster University | CSIR-CDRI

A58: Adipose derived protein, adipsin, regulates glucose and lipid uptake
by Joon Ho Moon | Yun Hye Kim | Ju Hee Lee | Jeongah Yoo | Joe Eun Son | Eashita Das | Florine Lenglin | Je Yuan Wang | James An | Sarah Kim | Weikang Cai | Chi-Chung Hui | Kyoung-Han Kim | C. Ronald Kahn | Hoon-Ki Sung | Translational Medicine Program, The Hospital for Sick Children | Developmental & Stem Cell Biology Program, The Hospital for Sick Children | Section of Integrative Physiology and Metabolism, Joslin Diabetes Center & Department of Medicine, Harvard Medical School | Dept Laboratory Medicine and Pathobiology, University of Toronto

A59: Luman as a Novel Regulator of Lipid and Cholesterol Metabolism
by Brandon Smith | Tiegh Taylor | Jenna Penney | Marica Bakovic | Ray Lu | University of Guelph

A60: n-3 PUFA induced n-6 PUFA bioconversion controls alpha-1- adrenoceptor expression in mice.
by Jiayu (Daisy) Ye | Sanjoy Ghosh | UBC-Okanagan

A61: Crosstalk Between the Kidneys and Pancreas in Glucose Control
by Maria Fernanda Fernandes | Iman M'hiri | Phillip Marvyn | Robin E. Duncan | University of Waterloo
A62: Using phospho-specific antibodies to study the role of phosphorylation in the regulation of CTP:phosphocholine cytidylyltransferase
by Michael J. McPhee | Neale D. Ridgway | Department of Biochemistry & Molecular Biology, Dalhousie University | Department of Pediatrics, Dalhousie University

A63: Lack of endonuclease G reduces linoleic acid accumulation and protects the cardiac muscle from inflammation
by Jiayu (Daisy) Ye | Sanjoy Ghosh | UBC-Okanagan

A64: Functional studies of ABC sterol transporters in lipid-bilayer nanodiscs
by William Jennings | Bala M. Xavier | Aiman Zein | Jyh-Yeuan Lee | Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa

A65: Intermittent Fasting Improves AgeAssociated Metabolic Abnormalities by Rejuvenation of White Adipose Tissue
by Ju Hee Lee, Eashita Das, Yun Hye Kim, Joanna Yeung, Yanqing Jiang, Min-Ah Choi, Jae-Ryong Kim, Hoon-Ki Sung | Translational Medicine, The Hospital for Sick Children

A66: VEGF-A pathway regulates transendothelial lipid uptake
by Yun Hye Kim, Joe Eun Son, Hira Raheel, Ju Hee Lee, Eashita Das, Jae-Ryong Kim, Jin Gyoong Park, Chi-chung Hui, Andras Nagy, Philhan Kim, Warren Lee, So Young Park, Kyung-Oh Doh, Hoon-Ki Sung | The Hospital for Sick Children

A67: Renal Steatosis Causes Onset of Glucose Intolerance In the Absence of Kidney Inflammation
by Iman M'Hiri | Maria Fernanada Fernandes | Phillip M. Marvyn | Robin E. Duncan | Department of Kinesiology, University of Waterloo | McMaster University

A68: Protective effects of phyto-oxylipin falcarnol in intestinal and systemic inflammation
by Amanda Stefanson | Marica Bakovic | University of Guelph
43rd Annual
Canadian Lipoprotein Conference

ABSTRACTS

POSTER SESSION I

Friday, June 8th, 2018
12:00-1:30 PM
The Regulation of PCSK9 by Lipoprotein Interactions by Samantha Sarkar | Tanja Kosenko | Alex Foo | Natalie Goto | Thomas Lagace | University of Ottawa Heart Institute | University of Ottawa Heart Institute | University of Ottawa | University of Ottawa | University of Ottawa Heart Institute

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) is a secreted plasma protein that binds and promotes degradation of cell-surface low density lipoprotein (LDL) receptors in the liver, impairing clearance of LDL from the blood. Thus, PCSK9 inhibition has emerged as a therapeutic avenue for hypercholesterolemia. It is known that PCSK9 binds to apolipoprotein B on LDL, but it is unknown what the physical binding sites are on either protein and what the structural requirements are of the interaction. Through site directed mutagenesis and in-vitro LDL binding assays, we have investigated a surface-exposed cluster of sites for natural gain-of-function mutation on the PCSK9 prodomain (S127R, D129G and L108R). We have found that the natural mutations cause a loss of LDL binding dependent on the presence of the native residue rather than on the charge of the residue at the site. In addition, using density gradient ultracentrifugation, we have found in vitro evidence that PCSK9 associates with intermediate density lipoproteins, but not very low density lipoproteins. These results were reflected in the plasma of hyper-triglyceridemic patients, where PCSK9 appeared to associate with more triglyceride-rich lipoproteins. Past studies in cell culture suggest that LDL inhibits PCSK9 activity, and our results contribute to a better understanding of the mechanism of PCSK9-lipoprotein binding and how that may affect PCSK9 activity in plasma. Elucidation of how lipoproteins regulate PCSK9 activity will reveal new targets for cholesterol-lowering therapeutics.
The role of AMPK in the lipid disorder caused by Malaria infection in mice by George Eduardo Gabriel Kluck | Camila Hübner Costabile Wendt | Maria Fernanda Carvalho Araújo | Kildare Rocha Miranda | Georgia Correa Atella | Federal University of Rio de Janeiro | Federal University of Rio de Janeiro | Federal University of Rio de Janeiro | Federal University of Rio de Janeiro

Malaria is the most important neglected disease in humans. It has been reported in 91 countries, and in 2016, there were 216 million cases, with 446,000 deaths worldwide. Malaria is caused by Plasmodium spp. parasites, which are transmitted to people through the bites of infected Anopheles mosquitoes. Plasmodium has its own enzymatic machinery for lipid synthesis during the liver stage, but it loses its ability when in the intraerythrocytic stage. Hence, this parasite manipulates the host vertebrate lipid metabolism for its development and dissemination. However, the exact mechanism needs further elucidation.

In this work, Swiss mice infected with P. chabaudi presented an altered plasma profile, such as hypertriglyceridemia, hypocholesterolemia, hyperproteinemia, and hypoglycemia. In addition, the analysis of the lipid profile of infected liver showed an accumulation of the main lipid classes as triacylglycerol, free fatty acids, and free cholesterol. Interestingly, the white adipose tissue also presented a significant increase in triacylglycerol. The infection with P. chabaudi altered the gene and protein expression of key enzymes and transcription factors involved in lipid metabolism. AMPK, a master regulator of metabolic and energy homeostasis was also regulated by infection, presenting a lower phosphorylation level and lower activity in liver. The treatment with Metformin (AMPK activator) reversed all the infection-induced modulation over the lipid metabolism and decreased significantly the infection. Together, these results provide new and important information on the lipid metabolism regulation by the Plasmodium parasite and highlight Metformin as a potentially novel drug to be used in Malaria therapeutics management.
The Natural History of Phytosterolemia: Observations on its Homeostasis by David Mymin | Gerald Salen | Barbara Triggs-Raine | Darrel J Waggoner | Thomas Dembinski | Grant M. Hatch | University of Manitoba | Rutgers New Jersey Medical School | University of Manitoba | University of Chicago | University of Manitoba

Background and aims: Phytosterolemia is a rare genetic disease caused by mutation of the ABCG5/8 gene. Our aim was to elucidate the natural history and homeostasis of phytosterolemia.

Methods: We analyzed a Hutterite kindred consisting of 21 homozygotes with phytosterolemia assembled over a period of two decades all of whom carried the ABCG8 S107X mutation and were treated with ezetimibe.

Results: Most of these subjects were asymptomatic and devoid of clinical stigmata, and this, since they were ascertained primarily by a process of cascade testing, suggests that, relative to its true prevalence, phytosterolemia is a condition of low morbidity. All subjects have responded well to treatment with ezetimibe. Initial (pre-treatment) and post-ezetimibe levels of cholesterol and sitosterol were measured and percentage changes on ezetimibe were calculated. We found initial levels to be inversely related to subjects’ ages as were percentage responses to ezetimibe therapy. There was also a direct correlation between initial levels and percentage responses to ezetimibe. Hence on-treatment levels were very uniform.

Conclusion: This evidence of a link with age leads us to propose that an age-related change in cholesterol and sterol homeostasis occurs at puberty in phytosterolemia and that the change is due to high sterol and/or stanol levels causing feedback inhibition of sterol regulatory element-binding protein (SREBP-2) processing. This would explain the well-documented phenomenon of depressed cholesterol synthesis in phytosterolemia. It is also well-known that LDL-receptor activity is increased and this feasibly explains reduced LDL levels and consequent reduction of plasma cholesterol and sitosterol levels. Downregulated SREBP-2 processing would be expected to also lower proprotein convertase subtilisin/kexin type 9 (PCSK9) levels and this would explain high LDL-receptor activity. The above state could be termed disrupted homeostasis and the alternative, seen mostly in children and characterized by hypercholesterolemia and hypersterolemia, simple homeostasis.
The role of tafazzin and monolysocardiolipin acyltransferase-1 in mitochondrial function in Barth Syndrome lymphoblasts by Edgard Mejia | Hana Zegallai | Eric Bouchard | Versha Banerji | Amir Rivandi | Grant M. Hatch | University of Manitoba | University of Manitoba | Université de Saint-Boniface | University of Manitoba | St. Boniface Hospital Research Center | University of Manitoba

**Background:** The mitochondrial polyglycerophospholipid cardiolipin (CL) is remodeled to obtain specific fatty acyl chains. This is predominantly accomplished by the transacylase enzyme tafazzin (TAZ). Barth Syndrome (BTHS) patients with TAZ gene mutations exhibit impaired TAZ activity and loss in mitochondrial respiratory function. Previous studies identified monolysocardiolipin acyltransferase-1 (MLCL AT-1) as a mitochondrial enzyme capable of remodelling CL with fatty acid. **Objectives:** In this study, we analyzed what relationship, if any, exits between TAZ and MLCL AT-1 with regard to CL remodeling and if transfection of BTHS lymphoblasts with a MLCL AT-1 expression construct improves mitochondrial respiratory function. **Results:** In healthy lymphoblasts reduction in TAZ expression through TAZ RNAi transfection resulted in a compensatory increase in MLCL AT-1 mRNA, protein and enzyme activity but CL mass was unaltered. In contrast, BTHS lymphoblasts exhibited decreased TAZ gene and protein expression but in addition decreased MLCL AT-1 expression and CL mass. Transfection of BTHS lymphoblasts with MLCL AT-1 expression construct increased CL, improved mitochondrial basal respiration and protein leak and decreased the proportion of cells producing superoxide but did not restore CL molecular species composition to control levels. In addition, BTHS lymphoblasts exhibited higher rates of glycolysis compared to healthy controls to compensate for reduced mitochondrial respiratory function. Mitochondrial supercomplex assembly was significantly impaired in BTHS lymphoblasts and transfection of BTHS lymphoblasts with MLCL AT-1 expression construct did not restore supercomplex assembly. **Conclusions:** The results suggest that expression of MLCL AT-1 is dependent on functional TAZ in healthy cells. In addition, transfection of BTHS lymphoblasts with a MLCL AT-1 expression construct compensates, but not completely, for loss of mitochondrial respiratory function.(Supported by grants from the Heart & Stroke Foundation of Canada and the Barth Syndrome Foundation Canada/USA)
Whole body knockdown of Mixed Lineage Kinase domain-Like protein decreases circulating lipids but does not protect against atherosclerosis by Adil Rasheed | Michele Geoffrion | My-Anh Nguyen | Richard Lee | Katey J. Rayner | University of Ottawa Heart Institute | University of Ottawa Heart Institute | University of Ottawa Heart Institute | Ionis Pharmaceuticals | University of Ottawa Heart Institute

Atherosclerosis is characterized by the formation of lipid-laden plaques in the aorta, a process which is initiated in part by monocyte infiltration. Monocyte-derived macrophages in the plaque become susceptible to cell death and release of their intracellular contents via the necroptotic signaling pathway. Mixed lineage kinase domain-like protein (MLKL) is a pseudokinase that represents the committed step of necroptosis. MLKL is phosphorylated and activated by upstream receptor-interacting serine/threonine-protein kinases 1 & 3 (RIPK1/3), which has been thought to solely result in MLKL oligomerization and insertion into the plasma and organelle membranes. However, MLKL can act as a scaffold protein critical for other cellular processes. To investigate the global role of MLKL in an atherosclerotic environment, 8 week old male and female Apoe-knockout (Apoe/-) mice were fed a Western diet for 8 weeks while receiving weekly subcutaneous injections of MLKL antisense oligonucleotides (ASOs) or the control ASO (50mg/kg). We confirmed the knockdown of MLKL at both the gene and protein level in the liver. The body weight of the mice receiving the MLKL ASOs was unchanged compared to those receiving control ASO. Furthermore, serum ALT and AST values were normal in all groups, confirming a lack of hepatotoxicity by the ASOs. H&E staining of aortic sinus sections revealed a trend towards an increase in lesion area in the Apoe/- mice receiving either MLKL ASO compared to controls. Surprisingly, serum cholesterol and triglycerides were both decreased by ~30% and ~40%, respectively (P<0.05), upon treatment with the MLKL ASOs. In the liver, there were no changes in cholesterol, while hepatic triglycerides trended towards a decrease with MLKL ASO treatment. These results indicate that MLKL plays a surprising role in regulating lipid homeostasis during high cholesterol feeding. Furthermore, despite decreases in circulating lipids, whole body knockdown of MLKL did not confer protection against atherosclerosis.
Functional analysis of novel PCSK9 natural mutants from Sapiens (R96L, R105W and P174S) and Denisovan (H449L) by Sepideh Mikaeeli | Emmanuelle Girard | Delia Susan Resiga | Josee Hamelin | Ali Ben Djoudi Ouadda | Nabil G. Seidah | IRCM, Université de Montréal, Experimental medicine, McGill university, Montreal, QC, Canada | IRCM, Université de Montréal, Montreal, QC, Canada | IRCM, Université de Montréal, Montreal, QC, Canada | IRCM, Université de Montréal, Montreal, QC, Canada | IRCM, Université de Montréal, Montreal, QC, Canada | IRCM, Université de Montréal, Montreal, QC, Canada

Objective: PCSK9 (Proprotein Convertase Subtilisin Kexin 9) is a major regulator of LDLc (Low Density Lipoprotein-cholesterol) via its ability to enhance the degradation of the LDLR (LDL receptor). It is now a major therapeutic target to reduce LDLc. Natural missense mutations in PCSK9 exons often result either in a GOF (gain-of-function) or LOF (loss-of-function) by modulating its activity on the LDLR. Herein, we analyzed in cell lines the activity of PCSK9 and that of three novel natural mutations found in the prodomain of PCSK9 (R96L, R105W) reported in Chinese FH ((Familial Hypercholesterolemia) patients and in the PCSK9 catalytic subunit (P174S) in Tunisian families. The fourth one is an archaic Denisovan mutation (H449L) in the hinge region separating the catalytic and CHRD domain (C-terminal Cys-His-rich domain). Methods: Mutations were generated using site-directed mutagenesis. In order to assay the extracellular and intracellular pathways of PCSK9-induced degradation of the LDLR we incubated hepatic Huh7 and IHH cells with media obtained from HEK293 cells overexpressing WT (wild type) and mutant PCSK9, as well as co-transfected hepatic cells with cDNAs encoding PCSK9 or its mutants with that of the LDLR. This was followed by functional assays including Elisa, Western blot, Di-LDL internalization and immunocytochemistry. Results: The preliminary results of our work showed a LOF activity for R96L and P174S mutations and a partial LOF activity for R105W mutation intracellularly. Also in the extracellular pathway the effect of R96L mutation followed the same LOF trend as the intracellular function. The H449L mutation did not significantly affect PCSK9 function in either pathway. Conclusion: The LOF-PCSK9-R96L (partially -R105W) and -P174S likely compensate the LDLc-raising in FH patients from Tunisia presenting a LOF-LDLR-D266N or from China, respectively. The unique Denisovan H449L mutation does not affect PCSK9 activity.
Effects of acutely elevated mitochondrial cholesterol by Luke Hattie | Barbara Karten | Dalhousie University, Department of Biochemistry and Molecular Biology | Dalhousie University, Department of Biochemistry and Molecular Biology

Increased mitochondrial cholesterol induces mitochondrial dysfunction and may be involved in the pathology of several disease states, including cancers and neurodegenerative disorders. The mechanisms by which cholesterol induces mitochondrial dysfunction have not been fully elucidated.

Previous methods for studying the effects of increased mitochondrial cholesterol have mostly relied on disease models, which may confound results. This study explores a new method for acutely and specifically increasing mitochondrial cholesterol by inducing the translocation of a cytosolic cholesterol transporter (STARD4) to mitochondria. Cytosolic proteins can be redirected to the mitochondria via attachment of rapamycin binding domains to a mitochondrial targeting sequence and a cytosolic protein, leading to translocation upon the addition of rapamycin. Using this system to target STARD4 to the mitochondria, cholesterol trafficking can be redirected to the mitochondria. This method allows acute induction of increased mitochondrial cholesterol and separation of this effect from the context of a disease-based system.

This system will be used to investigate metabolic consequences of increased mitochondrial cholesterol, including glucose metabolism and mitochondrial morphology. In the future, this system will be used to examine differential protein associations of the voltage-dependent anion channel, which has been suggested to participate in cholesterol-dependent mitochondrial dysfunction.
Impaired hepatic phosphatidylcholine synthesis leads to cholestasis in mice by Sereana Wan | Folkert Kuipers | Rick Havinga | Dennis E. Vance | Rene L. Jacobs | Jelske Van der Veen | University of Alberta | University Medical Center Gronigen | University of Alberta | University of Alberta

Phosphatidylethanolamine N-Methyltransferase (PEMT) is a hepatic, integral membrane protein localized to the endoplasmic reticulum that catalyzes ~30% of hepatic phosphatidylcholine (PC) biosynthesis. Pemt⁻/⁻ mice fed a high fat diet (HFD) rapidly develop steatohepatitis. PC is critical for maintaining membrane integrity. Interestingly, portions of the ER that are located in close proximity to the canalculus are enriched in PEMT. We hypothesized that PEMT activity is critical for biliary secretion during high fat feeding.

Pemt⁻/⁻ mice fed a HFD for 10 weeks developed cholestasis - i.e., elevated plasma bile acid (BA) concentrations and decreased biliary secretion rates of BA and PC. Total hepatic BSEP protein, responsible for BA secretion, was decreased in Pemt⁻/⁻ mice and appeared to be retained intracellularly. Electron microscopic evaluation showed that the canalicular membranes of Pemt⁻/⁻ mice contained fewer invaginations and displayed a smaller surface area than Pemt⁺/⁺ mice. Choline supplementation prevented the development of cholestasis: when Pemt⁻/⁻ mice were fed the HFD for 6 weeks to induce cholestasis and then switched to a choline-supplemented HFD for a further 6 weeks, cholestasis was reversed with normalization of plasma BA concentrations.

Reduced PC availability in the liver due to PEMT deficiency enhances the susceptibility to develop cholestasis. We propose that dietary choline supplementation might be a potential non-invasive therapy for specific cholestasis patients.
Large-scale deletions of the ABCA1 gene in patients with hypoalphalipoproteinemia by Jacqueline Dron, Jian Wang, Amanda Berberich, Michael Iacocca, Henian Cao, Ping Yang, Joan Knoll, Karine Tremblay, Diane Brisson, Christian Netzer, Ioanna Gouni-Berthold, Daniel Gaudet, Robert Hegele, Robarts Research Institute, Western University, Robarts Research Institute, Western University, ECOGENE-21, University of Montreal, ECOGENE-21, University of Montreal, University of Cologne, University of Cologne, ECOGENE-21, University of Montreal, Robarts Research Institute, Western University

Large-scale deletions and insertions, namely, copy-number variation (CNVs) have been studied in the context of lipid phenotypes and dyslipidemias, such as familial hypercholesterolemia, but have not been considered for extremes of high-density lipoprotein (HDL) cholesterol. We screened our collection of patients with low HDL cholesterol for CNVs disrupting genes consistent with the phenotype, such as ABCA1, APOA1, and LCAT. CNVs were detected using the VarSeq-CNV® caller algorithm, which relies on sequencing depth of coverage for analysis; we applied this algorithm to the sequencing data that had been generated previously by our targeted next-generation sequencing panel, LipidSeq. We identified four individuals who carried one of three unique deletions in ABCA1: a heterozygous deletion of exon 4, a heterozygous deletion spanning exons 8-31, and a heterozygous deletion of the entire ABCA1 gene. Breakpoints were confirmed for the smaller two deletions using Sanger sequencing, and the full-gene deletion was confirmed using the Affymetrix CytoScan™ HD Array. Given the role of the ABCA1 transporter in reverse cholesterol transport, large-scale disruptions of the gene likely lead to the protein’s loss of function, in which cholesterol efflux from macrophages is decreased, and the number of circulating HDL particles decreases as a result. This screening exercise serves as a proof-of-concept regarding this novel method of CNV identification using next-generation sequencing data in dyslipidemia patients—specifically, those with extremely low HDL cholesterol. This is the first example of ABCA1 CNVs in patients with hypoalphalipoproteinemia. While the prevalence of CNVs was low, our findings emphasize the genetic complexities underlying deviations in HDL cholesterol levels, and the value in considering large-scale variation when studying the genetic basis of extreme HDL cholesterol levels.
Effects of intervention with elongation factor 1A1 inhibitor, didemnin B, on NAFLD in western diet-induced obese mice by Rachel Wilson, Cynthia Sawyez, Brian Sutherland, Richard Zhang, Taylor Woolnough, Nica Borradaile | Department of Physiology and Pharmacology, Western University, London, Ontario, Canada

NAFLD is characterized by hepatic lipid accumulation, which can cause hepatocyte injury through lipotoxicity. This elicits inflammation and fibrosis, predominantly mediated by monocytes/macrophages and activated hepatic stellate cells (HSteC). We previously showed that the protein elongation factor EEF1A1 is induced in hepatocytes exposed to palmitate, downstream of ER stress. Furthermore, inhibition of EEF1A1 activity with the peptide didemnin B (DB) decreased palmitate-induced hepatocyte death in vitro, and decreased hepatic ER stress in vivo. However, the effects of EEF1A1 inhibition on inflammation and fibrosis in NAFLD are unknown. We hypothesized that inhibition of EEF1A1 activity with DB would decrease contributions of monocytes/macrophages and HSteC to inflammation and fibrosis, respectively, in NAFLD. In vitro, DB (80 nM) decreased monocyte proliferation (3-fold; p<0.0001), decreased monocyte differentiation to macrophages, and decreased HSteC proliferation (4-fold; p<0.0001) under lipotoxic conditions. These effects were primarily cytostatic, based on assays of LDH release and caspase-3 activity. To investigate the effects of intervention with DB on established, obesity-associated NAFLD in vivo, mice fed western diet for 24 weeks were administered i.p. injections of DB (50 ug/kg) once every 3 days for 14 days. Treatment with DB decreased liver triglycerides (1.5-fold, p=0.0011), plasma triglycerides (1.7-fold; p<0.0001), and plasma insulin (1.8-fold, p=0.0089), and improved oral glucose tolerance (1.1-fold, p=0.0081), with no effects on food consumption or epididymal fat mass. Additionally, histological analysis of liver fibrosis revealed a trend toward decreased total collagen area in mice treated with DB. Histological grading of NAFLD and immunohistochemical assessments of liver inflammation are ongoing. Hepatic ER stress, apoptosis, fibrosis and inflammation gene and protein expression will also be assessed. These analyses will elucidate the effects of EEF1A1 inhibition on liver inflammation and fibrosis and may highlight the therapeutic potential of partial protein synthesis inhibition in the treatment of NAFLD.
Effects of low dose niacin and vitamin D on vascular regeneration under lipotoxic conditions by Kia Peters | Zengxuan Nong | Chanho Park | Richard Zhang | Rachel Wilson | Hao Yin | Brian Sutherland | Cynthia Sawyez | J. Geoffrey Pickering | Nica Borradalle | Department of Physiology and Pharmacology, Western University | Robarts Research Institute | Western University | Western University | Western University | Robarts Research Institute | Robarts Research Institute | Western University | Robarts Research Institute | Department of Physiology and Pharmacology, Western University

Niacin and vitamin D have been shown to improve endothelial function and vascular regeneration in lean rodent models of vascular injury, ischemia, and metabolic disease. Here we tested whether niacin and vitamin D, alone or in combination, could directly improve endothelial cell angiogenic function under lipotoxic conditions, and improve revascularization and functional recovery in diet-induced obese mice with peripheral ischemia. Treatment with niacin (10 µM) or vitamin D (10 nM) alone improved human microvascular endothelial cell tube formation (1.5- and 1.4-fold, respectively) and stability (2.0- and 1.7-fold, respectively) (p<0.05). Transcriptomic analyses revealed that supplementation with either vitamin increased stress response and anti-inflammatory gene expression in the presence of high palmitate. However, vitamin D markedly decreased the expression of cell cycle regulators. Niacin, in contrast, altered the expression of miR-126-3p and miR-126-5p, which are known to regulate endothelial cell angiogenesis. To assess vascular regeneration in vivo, diet-induced obese mice with unilateral hind limb ischemic injury were treated once daily with niacin (50 mg/kg), or vitamin D (200 ng/kg), or both vitamins for 14 days. Niacin, but not vitamin D, improved recovery of hind limb use over the two week treatment period compared to vehicle (1.0-fold; p=0.014), as assessed by gait analyses. Histological analyses of tibialis anterior tissue sections revealed a trend toward a higher ratio of regenerating to non-regenerating myofibers with niacin treatment (p=0.13) compared to vitamin D. Vitamin D treatment was associated with metabolic disturbances including elevated plasma triglycerides and cholesterol, possibly indicating sustained inflammation. In summary, although both vitamins promoted in vitro angiogenic function of vascular endothelial cells, only niacin improved recovery of hind limb function following ischemic injury. Further understanding the effects of these vitamins on the vasculature may help guide therapeutic and nutritional recommendations for peripheral vascular disease in the setting of obesity.
Examination of the relative roles for oxysterol-binding protein homologues mediated non-vesicular and vesicular transport pathways in the subcellular distribution of phosphatidylserine. by Yanbo Yang

Phosphatidylserine (PS) is synthesized in the endoplasmic reticulum and enriched in the plasma membrane (PM). In the budding yeast S. cerevisiae, two oxysterol-binding protein homologues, Osh6p and Osh7p, have been characterized as soluble PS transfer proteins responsible for the non-vesicular transport and accumulation of PS in the PM. However, PS is also a constituent of secretory vesicles that originate from the Golgi apparatus and deliver lipids and proteins to the PM. Currently, it is unclear to what extent non-vesicular or vesicular transport pathways control the cellular distribution of PS or other lipids. Making use of a cellular probe for PS, GFP-Lact-C2, we examined the distribution of PS in a variety of deletion mutant strains of S. cerevisiae. The results indicated that in a small fraction of cells lacking both Osh6p and Osh7p have modest alterations in PS distribution. Conversely, accumulation of secretory vesicles via blockage in exocytosis revealed that secretory vesicles carry a considerable amount of PS en route to the PM and that this accumulation of PS is also independent of Osh6p and Osh7p. A high-content imaging based screen revealed that several proteins involved in vesicular trafficking pathways are required for proper PS distribution. In addition to mutants with defects in exocytosis, we also find that defects in the Golgi, endosomal maturation and retrograde transport pathways have altered PS distribution. Our results suggest that Osh6p and Osh7p may act as a fine-tuning mechanism or play a more specialized role rather than being the primary driver of PS transport.
Elevated Mitochondrial Cholesterol and its Effects on the formation of ER-Mitochondria Contact Sites. by Aaron Woblistin | Barbara Karten | Dalhousie University | Dalhousie University

Cholesterol is an essential component of all membranes in animal cells and regulates their fluidity and function. Different cellular membranes require different levels of cholesterol for optimal function. Mitochondrial cholesterol levels are normally very low, but they increase in some neurodegenerative diseases such as Alzheimer disease, during aging, and in many types of cancer cells. Cholesterol might influence mitochondrial function as well as ER-mitochondria contact sites (ER-MCS), which are important for calcium and lipid transport to the mitochondria.

In this study we are investigating the effects of elevated mitochondria cholesterol levels on mitochondrial function as well as ER-MCS. To evaluate mitochondria function we will measure mitochondria energy metabolism parameters. Further, we will use EM imaging to look at ER-MCS frequency and size as well as ER-mitochondria calcium and lipid transport for functional evaluation.
Molecular mechanisms of feedback inhibition of sphingolipid biosynthesis by Orm proteins by Omar Mourad | Gregory D. Fairn | University of Toronto | St. Michael's Hospital

Serine palmitoyltransferase (SPT) catalyzes the first committed step in de novo sphingolipid biosynthesis. Like many rate-limiting steps in biological pathways, it is subject to feedback inhibition. However, this inhibition is not due to the direct interaction of a sphingolipid intermediate with SPT. Instead, it is thought to be mediated by Orm proteins, a highly conserved family of integral endoplasmic reticulum proteins. Co-immunoprecipitation studies have shown that Orm proteins physically associate with SPT in a multiprotein complex referred to as the SPOTS complex in yeast. Genetic studies and metabolic analyses have shown that Orm proteins are required for the negative feedback loop and attenuation of SPT activity. However, the precise molecular mechanisms involved in the metabolite sensing and enzymatic inhibition remain unclear. We speculate that Orm proteins function in a two-step process, first binding a downstream metabolite such as ceramide and then via protein-protein interactions inhibit the SPT complex. Using a combination of random and alanine scanning mutagenesis we have generated several loss-of-function Orm1 proteins. While these proteins are stably expressed and localized to the ER, they can no longer act as a feedback inhibitor of the SPT complex. We are assessing the ability of these mutants to bind directly to ceramide in semi-intact cells and to interact with SPT. As aberrant regulation of the SPT is associated with asthma and hypertension, a better understanding of the Orm proteins may help with the development of interventions.
7-Ketocholesterol Impairs Phagocytosis and Efferocytosis via Dysregulation of Phosphatidylinositol 4,5-bisphosphate by Stella Lu | Gregory D. Fairn | Department of Biochemistry, University of Toronto and Keenan Research Centre for Biomedical Sciences, St. Michael’s Hospital | Department of Biochemistry, University of Toronto and Keenan Research Centre for Biomedical Sciences, St. Michael’s Hospital

The plasma membrane is inhomogeneously organized containing both highly ordered and disordered nanodomains. 7-Ketocholesterol (7KC), an oxysterol formed from the non-enzymatic oxidation of cholesterol, is a potent disruptor of membrane order. Importantly, 7KC is a component of oxidized LDL and accumulates in macrophage and foam cells found in arterial plaques. Using a murine macrophage cell line, J774, we report that both IgG-mediated and phosphatidylserine (PtdSer)-mediated phagocytic pathways are inhibited by the accumulation of 7KC. Examination of the well-studied Fcg Receptor pathway revealed that the cell surface receptor abundance and ligand binding are unaltered while downstream signaling and activation of Syk kinase is not affected. However, while the recruitment of phospholipase Cg1 (PLCg1) was unaffected its apparent enzymatic activity was compromised resulting in sustained phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P₂] levels and polymerized actin at the base of the phagocytic cup. Additionally, we found that 7KC prevented the activation of PLCb downstream of the P2Y₆ G-protein coupled receptor and that 7KC impaired PLCg activity in response to a direct elevation of cytosolic calcium induced by ionomycin. Finally, we demonstrate that 7KC partly attenuates the activity of rapamycin recruitable constitutively active PLCb3. Together, our results demonstrate that the accumulation of 7KC impairs macrophage function by altering PtdIns(4,5)P₂ catabolism and, thus, impairing actin depolymerization required for the completion of phagocytosis.
Arresting progression and promoting regression of atherosclerotic lesions: a dose-dependent effect of active immunization with an anti-glycosaminoglycan monoclonal antibody

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Background: Retention of apoB-containing lipoproteins by glycosaminoglycan-side chains of arterial proteoglycans is the key initiating step of atherosclerosis. Previously, we characterized the antiatherogenic properties of the anti-glycosaminoglycan chP3R99 mAb, which blocks subendothelial retention of LDL and induces a vaccine effect. This work was aimed at investigating the effect of chP3R99 mAb on different stages of atherosclerosis.

Methods: The impact of chP3R99 mAb on the onset and progression of atherosclerosis was assessed in apoE-/- mice treated with this mAb using different s.c. immunization schedules at beginning (preventive) and during disease development (therapeutic).

Results: Immunization with chP3R99 mAb (50 µg) reduced atherosclerotic lesions formation in preventive setting and arrested progression of established lesions at later stages of disease. This effect was associated to generation of anti-idiotype antibodies able to mimic glycosaminoglycan epitopes, thereby inducing anti-anti-idiotype antibodies, which recognized these polysaccharides. Intravenous infusion of IgG fraction from chP3R99-
immunized mice inhibited arterial retention and oxidation of infused LDL in apoE-/- mice. In agreement, preventive immunization with chP3R99 mAb significantly reduced infiltration of inflammatory macrophages whereas the accumulation of macrophages and T lymphocytes in advanced lesions were reverted with the therapeutic treatment. This effect was accompanied by a 3-fold increase in the IL-10/iNOS ratio, along with a reduction in circulating levels of IL-6. Accordingly, therapeutic immunization with a higher dose of chP3R99 mAb (200 µg) promoted regression of established atherosclerotic lesions in association with generation of higher levels of anti-glycosaminoglycan autologous antibodies.

**Discussion:** Targeting vascular glycosaminoglycans through an anti-idiotypic antibody cascade produced by chP3R99 mAb impaired subendothelial retention of LDL, attenuating the maladaptive inflammatory response. Consequently, chP3R99-immunization prevented lesion formation at early stages of atherosclerosis and arrested progression or even regressed established lesions at advanced stages of disease.

**Conclusion:** chP3R99-immunization against glycosaminoglycans is a promising novel strategy to intervene the atherogenic process at different stages.
Postprandial Lipid Dynamics and Intestinal GLP-1 Response in Healthy and Obese Adolescents by Victoria Higgins | Man Khun Chan | Khosrow Adeli | The Hospital for Sick Children, University of Toronto | The Hospital for Sick Children | The Hospital for Sick Children, University of Toronto

Background: Obesity and insulin resistance, becoming increasingly prevalent in adolescents, are commonly associated with dyslipidemia. Postprandial, rather than fasting, dyslipidemia independently predicts cardiovascular disease risk and is characterized by intestinal triglyceride-rich lipoprotein (TRL) overproduction. Co-secreted intestinal peptides, glucagon-like peptide 1 (GLP-1) and 2 (GLP-2), attenuate and paradoxically augment intestinal TRL output, respectively. We hypothesize that postprandial GLP-1 and GLP-2 responses are altered in obese adolescents with insulin resistance and/or postprandial dyslipidemia.

Methods: Normal weight (n=15; 8M/7F) and obese (n=15; 8M/7F) adolescents underwent an oral fat tolerance test, with blood collected at fasting and postprandially (1, 2, 4, 6 hours). The traditional lipid profile, glucose, and insulin were measured on the Abbott ARCHITECT. The lipoprotein phenotype profile was measured using nuclear magnetic resonance (NMR) spectroscopy. GLP-1 (active and total) and GLP-2 were measured by enzyme-linked immunosorbent assay (ELISA).

Results: Obese adolescents had significantly higher postprandial small high-density lipoprotein (HDL), small low-density lipoprotein (LDL), large TRL, very small TRL (remnant) particle number, and TRL-triglycerides. Postprandial active GLP-1 (area under the curve (AUC), incremental AUC (iAUC)) and total GLP-1 (iAUC) were significantly lower in obese compared to normal weight adolescents, suggesting a blunted GLP-1 response to fat ingestion. Furthermore, postprandial active GLP-1 significantly negatively correlated with degree of insulin resistance and postprandial triglycerides. Postprandial GLP-2 (iAUC) was also lower in obese adolescents, despite higher circulating concentrations.

Conclusion: The postprandial GLP-1 and GLP-2 response to a high-fat drink appears to be blunted in obese adolescents with postprandial dyslipidemia. However, it remains unknown if a blunted postprandial gut peptide response is a cause or consequence of the progression of these metabolic conditions in an obese state.
miR-130b modulates hepatic lipid metabolism and potently upregulates VLDL Production by Jing Zhang | Danielle Alvares | Ferdous Rastgar Jazii | Negar Khorosraviani | Lipei Liu | Khosrow Adeli | Hospital for Sick Children | Hospital for Sick Children; University of Toronto | Hospital for Sick Children; University of Toronto

A major complication of insulin resistant states is fasting dyslipidemia, a lipid profile characterized by elevated triglycerides (TG), increased small dense LDL and low HDL-cholesterol. This is primarily due to hepatic overproduction of VLDL-triglyceride and apolipoprotein B100 (apoB100). Recent studies from our laboratory has identified specific microRNAs that may directly induce changes in lipid homeostasis via changes in key regulators of lipid metabolism, such as microsomal triglyceride transfer protein (MTP) fatty acid synthase (FAS) and acyl-CoA carboxylase (ACC).

HepG2 cells, IHH cells and primary hamster hepatocytes underwent forward transfection with hsa-miR130b and/or inhibitor or negative control mimic. At the end of the treatment period, mRNA or protein was extracted, cells underwent radioactive metabolic labelling, or hepatic lipid droplets (LDs) were fluorescently imaged. Finally, chow-fed C57BL/6 mice were treated with once daily intravenous injection of miR-130b-3p agomir or negative control mimic for three days, and blood and tissue were collected.

Overexpression of miR-130b was found promote VLDL assembly and secretion in HepG2 and IHH cell models and increased the number or size of LDs. These effects may result from significant increases in mRNA levels of MTP and lipogenic genes such as ACC, FAS and SCD1. Concomitantly, increased MTP protein expression, and augmented TG secretion was observed in HepG2 cells. Conversely, overexpression of miR-130b-3p in primary hamster hepatocytes did not affect key regulators involved in lipogenesis or VLDL assembly or secretion. Acute administration of miR-130b-3p to chow-fed mice did not significantly alter plasma or VLDL- TG or cholesterol. Although there as no change in hepatic miR-130b-3p expression, a significant decrease in LDL receptor mRNA expression was observed. In all, these data suggest a potential role for miR-130b-3p in the regulation of hepatic lipid biosynthesis and mobilization in cultured hepatic cell lines that have defects in assembly and secretion of TG-rich lipoprotein particles.
Validation of the AMPK-SREBP2 pathway and implications on hepatic cholesterol homeostasis by Julia R.C. Nunes, Peyman Ghorbani, Morgan D. Fullerton | University of Ottawa Center for Infection, Immunity and Inflammation; Ottawa Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada.

The Canadian Liver Foundation states that 75% of the obese subpopulation are at risk for non-alcoholic fatty liver disease (NAFLD), with obesity being a major driver of the disease. Moreover, taken into consideration that 50% of Canadians are overweight, the impact of NAFLD should not be underestimated. The pathology is characterized by the excessive retention of lipids known as steatosis. These lipids (namely triglycerides) can arise from both dietary sources or de novo synthesis. NAFLD can progress to non-alcoholic steatohepatitis (NASH) which entails hepatocellular inflammation. A liver transplant becomes necessary once fibrosis occurs, and the individual's risk of developing hepatocellular carcinoma increases. Moreover, NAFLD is linked to characteristics of metabolic syndrome and has been identified as an independent risk factor for the development of cardiovascular disease. Cholesterol has also been implicated in NAFLD and is known for its cytotoxicity when in excess. SREBP2 is a master cholesterogenic transcription factor with auto-regulatory effects, that controls cholesterol metabolism. Amongst its target genes are the rate limiting enzyme HMGCR and a major receptor responsible for hepatic LDL uptake, LDLr. AMPK is a central cellular energy sensor that instigates catabolic and suppresses anabolic processes, such as cholesterol synthesis through the inhibition of HMGCR. Overall, pharmacological activation of AMPK has proven to be beneficial in the context of NAFLD. Various lines of evidence suggest that there are interactions between these two metabolic regulators; however, direct evidence is lacking. We hypothesize that AMPK can directly inhibit SREBP2 via phosphorylation thereby preventing its maturation and downstream signaling. This direct inhibition could reduce aberrant de novo cholesterol synthesis mediated by the upregulation of SREBP2 seen in NAFLD.
Hepatic cholesterol dysregulation by Ting Xiong |
Pei Yu | Paul Lebeau | Richard C. Austin | Bernardo L. Trigatti | McMaster University | McMaster University | McMaster University | McMaster University

Mice lacking the HDL receptor, SR-B1, and apolipoprotein E (SR-B1/ApoE dKO mice) exhibit hypercholesterolemia and spontaneous atherosclerosis spontaneously both in the aortic sinus and coronary arteries (CA) and develop myocardial infarction (MI), cardiac dysfunction and premature death by 8 weeks of age. In this study, we sought to investigate hepatic cholesterol regulation in SR-B1/apoE dKO mice as compared to ApoE KO littermate controls in order to explore factors driving altered plasma lipoprotein levels which may contribute to the unique CA atherosclerosis and MI phenotype in these mice.

SR-B1+/ApoE+/ (dKO) and littermate control SR-B1+/ApoE+/ (ApoE KO) mice were fed normal chow diet. A second cohort of dKO mice were treated daily with rosuvastatin (10 mg/kg i.v.) or saline for two weeks. All mice were analyzed at 5 weeks of age.

DKO mice exhibited reduced hepatic mature SREBP-2 protein levels compared to ApoE KO mice. Paradoxically, HMG-CoA reductase and PCSK9 mRNAs were elevated in livers of dKO mice compared to ApoE KO controls, while SREBP2 and LDLR mRNA levels were unchanged. Plasma PCSK9 protein levels were increased while hepatic LDLR protein levels were substantially reduced in dKO mice compared to ApoE KO controls. Upon treatment with rosuvastatin, hepatic LDLR and PCSK9 mRNA levels were substantially increased. Correspondingly, rosuvastatin treatment increased plasma PCSK9 protein levels, however hepatic LDLR protein levels were decreased.

Cholesterol homeostasis appears to be impaired in livers of SR-B1/ApoE dKO mice, which exhibit increased expression of some SREBP-2 regulated genes despite reduced mature SREBP-2 protein. Treatment with rosuvastatin increases in SREBP2 regulated genes. The outcome of rosuvastatin treatment, however, was reduced hepatic LDL R protein levels, likely a result of increased PCSK9 levels.
Phosphoethanolamine reverses characteristics of metabolic syndrome in Pcyt2(+/−) mice by Gabriela Mattos | Joy Oresajo | Dr. Marica Bakovic |
University of Guelph | University of Guelph | University of Guelph

Phosphatidylethanolamine (PE) is primarily synthesized by the CDP-ethanolamine branch of the Kennedy pathway, where a series of enzymatic reactions incorporate ethanolamine and diacylglycerol (DAG). This pathway is highly dependent on CTP-phosphoethanolamine cytidylyltransferase (ET/Pcyt2), which is the rate limiting enzyme. Pcyt2 heterozygous mice (Pcyt2(+/−)) accumulate DAG and triacylglycerol (TAG) due to the lack of utilization in the Kennedy pathway. This accumulation leads to the development of metabolic syndrome, specifically obesity, insulin resistance, and hepatosteatosis. Here, we demonstrate that two month supplementation with 1% phosphoethanolamine (PEA), the substrate of Pcyt2, has the ability to reverse the metabolic syndrome phenotype that is characteristic to the Pcyt2(+/−) mouse model. PEA supplementation improves hepatosteatosis and liver damage in Pcyt2(+/−) mice. PEA supplementation stimulates mitochondrial biogenesis, and improves insulin signalling within muscle and liver tissue, while also stimulating genes involved in phospholipid biosynthesis and lipid metabolism. We describe specific mechanisms for the reversion of Pcyt2(+/−) phenotype with PEA, as well as the potential role PEA may play in diabetes treatment.
Scavenger receptor class B type 1 knockout mice develop extensive diet-induced aortic sinus and coronary artery atherosclerosis in an age-dependent manner. by Samuel K Lee | Mark T Fuller | Bernardo L Trigatti | McMaster University | McMaster University

Objective: Coronary artery (CA) atherosclerosis is the most common type of cardiovascular disease. Knockout (KO) of scavenger receptor class B type 1 (SR-B1) in mice with atherogenic mutations results in aortic sinus and occlusive CA atherosclerosis, myocardial infarction (MI), and early death. However, the effects of age on CA atherosclerosis development in mice has not been examined. Here, we examined the effects of age on the degree of CA atherosclerosis and survival in SR-B1 single KO mice fed an atherogenic diet.

Methods: SR-B1 KO mice between 12-52 weeks old were fed a high-fat, high-cholesterol, cholate-containing diet for 20 weeks, or humanely euthanized when they exhibited ruffled coat, hunched posture, lethargy and labored breathing. Hearts were harvested and cryosectioned, and aortic sinus and CA atherosclerotic plaques were analyzed histologically.

Results: SR-B1 KO mice fed the diet for 20 weeks, starting at 12 weeks-of-age, did not exhibit signs of illness or early death. However, 24-52-week-old SR-B1 KO mice fed the diet displayed signs of illness and reached endpoint after 4-17 weeks, such that the feeding period required to reach endpoint was inversely proportional to their age at the start of the diet. The 12-week-old SR-B1 KO mice developed extensive aortic sinus atherosclerosis with occasional CA atherosclerosis, whereas the 24-52-week-old SR-B1 KO mice developed extensive CA atherosclerosis. Surprisingly, however, aortic sinus plaque sizes were substantially smaller than those of the 12-week-old SR-B1 KO mice. Conclusions: SR-B1 KO mice fed the atherogenic diet exhibit extensive CA atherosclerosis and reduced survival in an age-dependent manner. Furthermore, the levels of CA atherosclerosis do not correlate with the extent of aortic sinus atherosclerosis, suggesting that aortic sinus atherosclerosis may not be a good predictor of CA atherosclerosis. Overall, older-aged SR-B1 KO mice fed the atherogenic diet can be used as a model to analyze extensive CA atherosclerosis and MI.
The lysophosphatidic acid acyltransferases (acylglycerophosphate acyltransferases) family: one reaction, five enzymes, many roles by Ryan Bradley | Ashkan Hashemi | Emily Mardian | Juan Jose Aristizabal Henao | Darin Bloemberg | Joe Quadrilatero | Ken D. Stark | Robin E. Duncan | University of Waterloo | University of Waterloo | University of Waterloo | University of Waterloo | University of Waterloo | University of Waterloo | University of Waterloo | University of Waterloo | University of Waterloo

Lysophosphatidic acid acyltransferases (LPAATs)/acylglycerophosphate acyltransferases (AGPATs) are a homologous group of enzymes that catalyze the de novo formation of phosphatidic acid from lysophosphatidic acid (LPA) and a fatty acyl-CoA. Originally, eleven members of this family were identified, although only LPAATa-e remain as canonical LPAATs, synthesizing PA from LPA. Recent molecular studies suggest that individual LPAAT homologues produce functionally distinct pools of phosphatidic acid, whereas gene ablation studies demonstrate unique roles despite a similar biochemical function. Loss of the individual enzymes not only causes diverse effects on down-stream lipid metabolism, which can vary even for a single enzyme from one tissue to the next, but also results in a wide array of physiological consequences, ranging from cognitive impairment, to lipodystrophy, to embryonic lethality. We have previously characterized Lpaatd/Agpat4 as a mitochondrial LPAAT that is important in regulating the content of down-stream phospholipids such as PC, PE, and PI in the brain. However, recent data on Lpaatd-/- mice in our laboratory have now demonstrated that Lpaatd also plays several functionally distinct roles in other tissues, thus resolving the apparent redundancy of multiple members of this enzyme family. Results from three major studies on the characterization of Lpaatd-/- mice are discussed, demonstrating its importance in the brain, skeletal muscle, and epididymal and perirenal white adipose tissues. The discovery that Lpaatd can modulate different downstream glycerolipid pathways in different tissues now expands the appreciation for the diverse functional roles of this enzyme family. On the basis of recent findings, we propose that the individual LPAAT enzymes may synthesize functionally distinct pools of phosphatidic acid. We further propose that individual LPAATs play unique roles in the support of down-stream phospholipid and TAG biosynthetic pathways that can vary for the same enzyme in a tissue-specific, or even cell-specific manner.
Thermogenesis independent metabolic benefits conferred by intermittent fasting in ob/ob mice by Yun Hye Kim | Ju Hee Lee | Kyoung-Han Kim | The Hospital for Sick Children | The Hospital for Sick Children | University of Ottawa Heart Institute

Intermittent fasting (IF), a periodic caloric restriction regimen, was recently studied as an intervention method to combat obesity and diabetes. Our previous study showed that IF in mice leads to reduced body weight gain and improved insulin sensitivity (IS) via activation of non-shivering thermogenesis in white adipose tissue (WAT). Our preliminary studies suggest that in genetically obese (ob/ob) mice, metabolic benefits of IF are achieved in the absence of thermogenesis-induced browning. Thus, we anticipate that improvements in other key metabolic tissues (e.g. pancreatic beta cells and liver steatosis) may contribute to IF-mediated metabolic benefits.

Male ob/ob mice were used as late stage type 2 diabetes model and subjected to pair-fed isocaloric IF regimen. Histopathological analyses and immunofluorescent staining of pancreas beta cells and liver were performed in the ad libitum (AL) and IF animals. Plasma insulin levels were examined to assess insulin production. Future plans include ELISA/multiplex analysis to detect differences in Alanine Transaminase (ALT) activity as an indicator of liver damage.

Despite the absence of adipose thermogenic improvement in IF group, we observed a mild but significant improvement in glucose tolerance compared to the AL. Histological analysis of WAT also showed no browning differences, suggesting that metabolic benefits may be mediated by a thermogenesis-independent mechanism. Possible alternatives include the secretion of healthy adipokines or improved IS. As well, immunofluorescent images of the pancreas showed that islet size of IF animals were larger than the AL, indicating possible differences in insulin secretion.

To conclude, our studies show that ob/ob mice under the IF regimen may obtain metabolic benefits through a thermogenesis-independent manner, primarily due to changes in the pancreas. Our data propose IF as a therapeutic method to reverse late stage diabetes via beta cell activation/proliferation.
Mechanism and efficacy of statins as metastasis-prevention agents in breast cancer

by Jenna E van Leeuwen | Rosemary Yu | Joseph Longo | Cunjie Zhang |
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In early stage breast cancer (BC) treated with front line therapy, 15-20% will reoccur as distant metastases, accounting for nearly all BC-related deaths. Epidemiological studies consistently report that BC patients who take statins after front line therapy have 30-60% lowered risk of metastatic recurrence. Our work demonstrates that statins preferentially induced apoptosis in cancer cells that have undergone EMT. Mechanistically, statin treatment inhibited the metabolic biosynthetic pathway for dolichol, required for the expression of a class of N-glycans associated with EMT and metastasis, leading to cancer cell death while leaving normal cells intact. Using a mouse model of post-surgical metastatic BC, we showed that adjuvant statin treatment effectively delayed metastasis and improved survival. By demonstrating efficacy, elucidating mechanism, and identifying biomarkers of statin sensitivity, our work supports the immediate repurposing of this effective, safe, and inexpensive drug to target breast cancer metastasis.
Free Fatty Acids Liberated by Lipoprotein Lipase from Lipoproteins Inhibit Cholesterol Efflux by Activating Akt by Jenika D. Marshall | Bronwyn A. Woolfrey | Emily R. Courage | Madeline N. Fitzpatrick | Ryan F. Elliott | Yanbo Yang | Robert J. Brown | Memorial University of Newfoundland | Memorial University of Newfoundland | Memorial University of Newfoundland | Memorial University of Newfoundland | Memorial University of Newfoundland | Memorial University of Newfoundland | Memorial University of Newfoundland

Lipoprotein lipase (LPL) is upregulated in atherosclerotic lesions, and the products released from the hydrolysis of lipoprotein lipids promote disease. The mechanisms behind this process are not completely understood. Using antibody arrays, we previously showed that phosphorylation of the signaling molecule protein kinase B (or Akt) within THP-1 macrophages is increased after a 30 minute incubation with lipid hydrolysis products generated by LPL from total lipoproteins. The free fatty acid (FFA) component was responsible for this effect. We thus hypothesized that Akt activation may play a role in cellular lipid accumulation. Supporting this, we show that inhibition of phosphatidylinositol-3 kinase (which is upstream of Akt) in THP-1 macrophages treated with the total FFA component for 18 hours results in a significant 20% reduction of neutral lipid accumulation by Oil Red O staining. We first characterized the dose and time needed for Akt phosphorylation in response to total FFA; we showed that Ser\textsuperscript{473} is phosphorylated in a dose-dependent manner at 2 hours, and that Akt could remain phosphorylated up to 18 hours. We further tested the effects of different classes of FFA on Akt phosphorylation, and observed that the monounsaturated fatty acid component significantly increased Akt phosphorylation after 2 hours versus control cells. Palmitoleate was specifically responsible for the Akt phosphorylation. Using THP-1 macrophages, we replicated our previous study showing that the total FFA component significantly inhibits cholesterol efflux to apolipoprotein A-I. We further show that the inhibition of FFA-mediated Akt phosphorylation using the Akt inhibitor MK-2206 restores cholesterol efflux levels to those observed for apolipoprotein A-I alone. We are currently assessing the effect of palmitoleate on cholesterol efflux. Overall, our data support a negative role for LPL in macrophage lipid accumulation, such that the total FFA liberated by LPL leads to lipid accumulation and impaired cholesterol efflux via Akt activation.
Ethanolamine Transport and the Choline Transporter-like Protein 1 (CTL1) by Adrian Taylor

| Marica Bakovic | University of Guelph | University of Guelph

Ethanolamine is an essential precursor in the synthesis of ethanolamine containing phospholipids like plasmalogens and phosphatidylethanolamine (PE). These phospholipids are found in the cell membrane and contribute to its structural integrity. In addition, they regulate cell division, cell signalling and are also vital for maintaining mitochondrial morphology. However, because ethanolamine is not produced endogenously, it needs to be obtained from exogenous lipids in the diet. Moreover, ethanolamine does not readily diffuse across the plasma membrane to enter the cell and requires a transporter in order to traverse the plasma membrane. To date, a transporter for ethanolamine has not been identified but our lab has evidence that choline transporter-like protein 1 (CTL1) may also function as an ethanolamine transporter.
High Density Lipoprotein Mediated Protection of Macrophages against Apoptosis Requires Scavenger Receptor Class B Type 1 Activity and Sphingosine-1-Phosphate by Kevin Chathely | Bernardo Trigatti | McMaster University | Bernardo.Trigatti@taari.ca

BACKGROUND/OBJECTIVES: Prevention of macrophage apoptosis in advanced atherosclerotic lesions can help stop atherosclerosis progression to vulnerable plaques. High density lipoprotein (HDL) can protect macrophages from apoptosis that has been induced by a variety of agents. We hypothesize that this is the consequence of the sphingolipid, sphingosine-1-phosphate (S1P), specifically carried by HDL, and transferred to S1P receptor 1 (S1PR1) on the cells via the HDL receptor, scavenger receptor class B type 1 (SR-B1). METHODS: Apoptosis was induced in murine peritoneal macrophages from wild type and different knockout mice with the ER stress inducing agent tunicamycin. Apoptosis was then observed and detected by terminal deoxynucleotidyl transferase mediated dUTP nick end labeling through fluorescent microscopy. All experiments were conducted with an n of 3 or 4. RESULTS: Treatment of cells with HDL protected them against tunicamycin induced apoptosis. In contrast, pre-treatment of HDL with S1P lyase, which irreversibly cleaves S1P, eliminated the ability of HDL to protect macrophages. Furthermore, HDL-dependent protection of macrophages against apoptosis required both the HDL receptor SRB1 and the S1PR1. Inhibitor of SRB1’s lipid transport activity also prevented HDL dependant protection against apoptosis. CONCLUSIONS: These results suggest that the HDL mediated protection of macrophages against apoptosis may involve SRB1 mediated delivery of S1P from HDL to the S1PR1. Understanding the mechanisms by which HDL elicits atheroprotective signalling in macrophages will provide insight into new targets for therapeutic intervention.
High levels of plasma low-density lipoprotein cholesterol (LDL-C) are a main risk factor for the development of coronary artery disease. Plasma LDL-C is controlled by uptake and degradation in the liver, which is facilitated by the LDL receptor (LDLR). LDLR protein levels are negatively affected by proprotein convertase subtilisin/kexin-9 (PCSK9), a secreted protein that interacts with LDLR at the plasma membrane, initiating uptake and degradation of both proteins in the lysosome. PCSK9 binds to an epidermal growth factor (EGF)-like domain (EGF-A) of LDLR, and Ca2+ coordination in the EGF-A domain is required for this interaction. Injectable antibodies have been developed to bind PCSK9, inhibiting association with LDLR and significantly lowering LDL-C in clinical trials. Unfortunately, the cost of therapy makes this treatment impractical for widespread adoption. Our laboratory has identified an alternative mechanism of PCSK9 and LDLR interaction dominant in fibroblasts. In these cells, PCSK9 dissociates from LDLR in early endosomes, allowing LDLR to recycle to the cell surface while PCSK9 is degraded in the lysosome. We hypothesize that this phenomenon can be attributed to reduced Ca2+ levels in the fibroblast early endosome, triggering release of Ca2+ from the EGF-A domain and subsequent dissociation of PCSK9. Stimulating this mechanism in liver should increase LDLR at the cell surface, thereby boosting LDL-C and PCSK9 clearance. We are currently working to validate this premise by performing in vitro studies with LDLR mutants that can bind PCSK9 in the absence of Ca2+. Moreover, we have developed several knockout cell lines using CRISPR technology to evaluate the role of proteins potentially involved in LDLR trafficking in PCSK9 sensitive and resistant tissues.
Regulation of Lipid Homeostasis by Glucocorticoids in the Mediobasal Hypothalamus by Emilie Beaulieu-Bayne | Jennifer Lee | Jessica Yue |

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Hypertriglyceridemia, resulting from elevated hepatic secretion of triglyceride-rich very-low-density lipoproteins (VLDL-TG), is associated with metabolic diseases such as obesity and diabetes. Additionally, excessive levels and/or action of glucocorticoids (GCs) are also associated with obesity and dyslipidemia. Whereas the peripheral effect of GCs to elevate lipemia is well known, less is known about the effect of GCs acting in the brain to modulate metabolism. Since the brain senses nutrients and hormones to regulate peripheral lipid homeostasis, the aim of this study is to delineate a mechanism of GC action in the mediobasal hypothalamus (MBH) that modulates lipid metabolism in normal and pre-obese rodents. We hypothesize that GCs act on GC receptors (GRs) in the MBH to modulate lipid homeostasis via increased plasma TG and hepatic VLDL-TG secretion. Male Sprague Dawley rats underwent stereotaxic MBH bilateral cannulation and intravenous (iv) and intraarterial catheterization to allow for simultaneous direct infusions into the MBH, iv infusion of poloxamer, and blood sampling. We studied the effects of MBH dexamethasone (DEX), a synthetic GC, on hepatic lipid secretion in both regular chow (RC) and high fat diet (HFD)-fed rats. We demonstrate that MBH DEX increases the rate of hepatic TG secretion compared to MBH vehicle controls. This effect is mediated by GRs since co-infusion of GR-antagonist mifepristone, as well as chronic inhibition of GR in the MBH by GR shRNA, negates the MBH GCs lipostimulatory effect. Basal plasma corticosterone levels and hepatic TG secretion are elevated in 3-day HFD-fed rats independently of changes in body weight or fasting blood glucose. MBH DEX and HFD are associated with alterations of certain hepatic lipogenic enzymes. Thus, we demonstrate for the first time that MBH GC action modulates hepatic TG secretion. Follow-up studies will test whether targeted inhibition of MBH GC action improves lipid regulation in obesity-related metabolic disease.
Studies of effects of LysMcre/creS1P1lox/lox mutations on S1P1 expression in different tissues of atherogenic mice by Narmada Thyagarajan | Leticia Gonzalez | Darren Sam | Usama Tahir | Bernardo Trigatti | McMaster University | McMaster University | McMaster University | McMaster University

Sphingosine 1 phosphate, a bioactive lysosphingolipid, promotes anti-atherogenic properties in different cell types by signaling via one of the G-protein coupled receptors termed S1P receptor type 1 (S1P1). Others have reported that treatment of either apoE−/− or LDLR−/− mice with the broad spectrum S1P receptor agonist FTY720 or selective S1P1 agonists reduces atherosclerosis. We have previously reported that activation of S1P1 signaling in macrophages triggers chemotaxis and protects them against apoptosis induction, and that myeloid selective inactivation of S1P1 achieved by transplanting bone marrow from LysMcre/creS1P1lox/lox mice into LDLR−/− recipients increased high fat diet-induced atherosclerosis, necrotic core development and plaque apoptosis. Surprisingly, however, LysMcre/creS1P1lox/lox apoE−/− mice generated by crossing LysMcre/creS1P1lox/lox mice with apoE−/− mice exhibited dramatically attenuated atherosclerosis compared to control LysMcre/creS1P1wt/wt apoE−/− mice. To begin to understand the discrepancy between the effects of the global versus the bone marrow selective LysMcre/creS1P1lox/lox mutation, we examined S1P1 mRNA levels in different tissues of LysMcre/creS1P1lox/lox apoE−/− and control LysMcre/creS1P1wt/wt apoE−/− mice using RT-PCR. S1P1 mRNA was reduced by 60% (P=0.01) in brains and 40% (P=0.006) in kidneys and appeared to be lower in aortas and spleens (not statistically significant) of LysMcre/creS1P1lox/lox apoE−/− compared to LysMcre/creS1P1wt/wt apoE−/− mice (n=3). These findings suggest that reductions in S1P1 levels in tissues/cell types other than bone marrow derived myeloid cells may explain the discrepancy between atherosclerosis results in the mice carrying the global versus bone marrow specific LysMcre/creS1P1lox/lox mutations.
Portal vein GLP-1 receptor activation modulates dietary fat absorption and intestinal lipoprotein metabolism by Simon Hoffman | Khosrow Adeli | Molecular Medicine, Research Institute, The Hospital for Sick Children and University of Toronto, Toronto, Ontario, M5G 1X8, Canada | Molecular Medicine, Research Institute, The Hospital for Sick Children and University of Toronto, Toronto, Ontario, M5G 1X8, Canada

Post-prandial lipid metabolism becomes altered during insulin resistance and type 2 diabetes (T2D), resulting in overproduction of intestinal lipoproteins, and elevated plasma triglyceride (TG) levels; precursors to the development of cardiovascular disease (CVD). Recently, our laboratory has demonstrated that the gut-derived hormone glucagon-like peptide (GLP)-1 can attenuate lipoprotein production through a complex gut-brain-liver axis. Since intestinally-derived GLP-1 is rapidly degraded in the blood, it is likely GLP-1 acts in the portal venous bed. Therefore, the objective of this study was to examine the effect an acute dose of GLP-1 would have on intestinal lipoprotein metabolism if injected directly into the portal vein. To assess this, Syrian golden hamsters underwent jugular cannulation, then the portal vein was injected with active GLP-1 peptide (7-36) (100uL of 0.01ug/uL) (n=5), or vehicle (100uL; PBS) (n=5). In addition, one group was pre-treated with the GLP-1R antagonist exendin-9-39 (100ul of 0.05ug/uL) (n=5) prior to portal vein GLP-1 injection. Finally, a subset of animals received an i.v. infusion of GLP-1 (n=4) though their jugular catheter. After treatment, animals received a fat load, and an i.v. infusion of triton to prevent lipoprotein clearance. Postprandial TG accumulation was assessed by blood draws over a 6h period, and triglyceride-rich lipoprotein (TRL) fractions were isolated by ultracentrifugation. Hamsters who received a portal injection of GLP-1 showed markedly decreased postprandial TG accumulation in both whole plasma and TRL fractions relative to vehicle controls. This effect was abrogated by exendin-9-39 pre-treatment. Importantly, hamsters which received a jugular infusion of GLP-1 did not exhibit decreases in TG accumulation, suggesting GLP-1R activation in the area of the portal bed is requisite to elicit this effect. Overall, we present novel evidence supporting the site-specific activity of GLP-1; wherein, its ability to reduce postprandial lipemia relies on GLP-1R-containing neurons in the portal venous bed.
The Scavenger Receptor Class B Type I regulates the levels of the Sphingosine 1-Phosphate Receptor 1 in mouse peritoneal macrophages by Christine Bassila | Bernardo Trigatti | McMaster University | McMaster University

High-density lipoprotein (HDL) inhibits atherosclerosis and reverses the accumulation of macrophage foam cells in atherosclerotic plaques by triggering cholesterol efflux from macrophages and activating various signaling pathways leading to atheroprotective cellular responses. HDL-induced signaling in macrophages is mediated by the scavenger receptor class B type 1 (SR-B1). This receptor interacts with a G-protein coupled receptor, the sphingosine-1-phosphate receptor 1 (S1PR1), in presence of S1P. FTY720, a known pro-agonist of S1PRs, was found to trigger the HDL-stimulated macrophage migration in peritoneal macrophages from wild type (WT) mice but not in macrophages from SR-B1 deficient mice.

To understand why FTY720 was unable to stimulate the migration of SR-B1 KO macrophages, we investigated the protein and the mRNA levels of S1PR1 in peritoneal macrophages prepared from SR-B1 KO mice compared to WT mice. We also examined the ability of the S1PR1 selective agonist, SEW-2871, to activate signal transduction pathways involving protein kinase B (Akt) and the extracellular signal-regulated kinase (ERK 1/2) in peritoneal macrophages collected from WT or SR-B1 KO mice.

Immunoblot analysis demonstrated a significant reduction in S1PR1 protein levels in macrophages from SR-B1 KO compared to WT mice. The S1PR1 selective agonist, SEW2871, induced significant phosphorylation of AKT and ERK 1/2 in SR-B1 deficient macrophages compared to control WT macrophages.

Our findings suggest that SR-B1 may play an essential role in controlling the steady state levels of S1PR1 in macrophages.
Depletion of lesion macrophages characterizes atherosclerosis regression induced by naringenin by Amy C. Burke | Brian G. Sutherland |
Dawn E. Telford | Marisa R. Morrow | Cynthia G. Sawyez | Jane Y. Edwards | Murray W. Huff |
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In mouse models, diet-induced metabolic dysregulation (MetS) and atherosclerosis can be reversed by intervention with a low-fat diet. Supplementation of a high-fat (HFHC) diet with the flavonoid naringenin prevents abnormal lipid and glucose metabolism, and attenuates atherosclerosis. In the present study, we hypothesized that intervention by the addition of naringenin to a chow diet would enhance atherosclerosis regression and reversal of MetS. Ldlr⁻/⁻ mice were fed a HFHC diet for 12 weeks to induce MetS and intermediate atherosclerosis (baseline). Intervention for an additional 12 weeks consisted of transfer to: 1) a low-fat, chow diet, 2) chow with 3% naringenin (Nar+chow) or 3) continuation on the HFHC diet. Hypercholesterolemia and hypertriglyceridemia induced by the HFHC diet were reversed to a greater extent by intervention with Nar+chow (-105% and -124%) compared to chow (-96% vs -103%), suggesting Nar+chow may enhance atherosclerosis regression. Relative to baseline, aortic cholesteryl ester (CE) increased with chow alone (+40%), whereas Nar+chow reversed aortic CE (-19%). Compared to baseline, aortic sinus plaque size increased with chow (+47%), whereas with Nar+chow plaque size increased only 14%, indicating almost complete attenuation of lesion growth. Nar+chow reduced lesion macrophages by 73%, while the reduction with chow alone was only 53%. Mechanistically, intervention with Nar+chow enhanced the reduction in elevated total and Ly6C<sup>hi</sup>blood monocytes (-63% and -76%) compared to chow (-37% and -41%). Reduced necrotic area (6.5% vs 7.5%, trend) resulted from Nar+chow intervention, and both intervention diets decreased apoptotic cells similarly. Nar+chow reversed body weight (-93%) to a greater extent than chow (-60%), induced a larger decrease in liver triglycerides (-110% vs -86%), and enhanced reductions in fasting blood glucose, plasma insulin and insulin sensitivity. In conclusion, intervention with the addition of naringenin to a chow diet, enhanced reversal of metabolic dysfunction, halted progression of atherosclerosis and improved lesion morphology.
Effects of hematopoietic deficiency of pro-apoptotic protein Bim on the development of atherosclerosis

Alexander Qian | Pei Yu | Leticia Gonzalez | Bernardo Trigatti | McMaster University | McMaster University

In advanced lesions, macrophage apoptosis contributes to plaque progression and necrotic core formation resulting in more vulnerable plaques prone to rupture and thrombotic events. The pro-apoptotic BH3-only protein Bim is involved in controlling cell death in T-cells, B-cells, macrophages and other leukocytes that contribute to atherosclerotic plaque progression. It has been reported that Bim mediates apoptosis in response to prolonged ER stress and oxidative stress in a variety of cells including macrophages. We hypothesized, knocking out Bim in hematopoietic cells including macrophages may reduce apoptosis and necrotic core formation within atherosclerotic plaques. To analyze the effects of Bim deficiency in bone marrow derived cells on development of atherosclerosis, we transplanted bone marrow (BM) from Bim\(^{-/-}\) and wild-type (WT) mice into irradiated 10 week old apoA1\(^{-/-}\) LDLR\(^{-/-}\) mice. Following a four-week recovery period, mice were fed a high-fat Western Diet (21% butterfat, 1% safflower oil, 0.15% cholesterol) for 10 weeks. Bim\(^{-/-}\) BM transplanted mice exhibited normal body and heart weights compared to WT BM transplanted mice, however they developed significantly larger spleens and increased circulating leukocytes. Mice transplanted with Bim\(^{-/-}\) BM also had significant reductions in plasma cholesterol levels and plasma triglyceride levels. Interestingly, in aortic root sections stained with ORO, we saw significant reductions in atherosclerotic plaque size and sizes of necrotic cores within those plaques in mice transplanted with Bim\(^{-/-}\) BM compared to mice transplanted with WT BM. These findings suggest that the pro-apoptotic protein Bim may play an important role in leukocytes in atherosclerotic plaque development.
Statins cause IL-1β-dependant adipose insulin resistance via lower protein prenylation by Brandyn Henriksbo | Joshua Xu | Jobanjit Phulka | Brittaney Duggan | Joe Cavallari | Kevin Foley | Akhilesh Tamrakar | Jonathan Schertzer | McMaster University | McMaster University | McMaster University | McMaster University | McMaster University | CSIR-CDRI | McMaster University

Statins are one of the most widely prescribed drug classes because they lower circulating low density lipoprotein-cholesterol (LDL-C) and reduce the risk of cardiovascular events. Statin-mediated inhibition of HMG-CoA reductase also lowers substrates required for protein prenylation. This cholesterol independent effect of statins can alter immune function. Lower protein prenylation can increase IL-1β. This pro-inflammatory cytokine can promote insulin resistance, which may be a factor in the recent evidence linking statins to increased incidence of diabetes. IL-1β is unique compared to most cytokines because it is regulated by the NLRP3 inflammasome. We have already shown that statins cause NLRP3-depdant insulin resistance in fat tissue, but it was not known if this is due to cholesterol, prenylation or IL-1β-mediated inflammation.

We hypothesized that statin-induced lowering of protein prenylation activates the NLRP3 inflammasome, which would cause IL-1β-dependent insulin resistance in fat tissue. Mouse white adipose tissue (WAT) explants were exposed to Atorvastatin (1uM, 18h) with LPS (4h) followed by stimulation with insulin and quantification of the phosphorylation status of AKT. We showed that atorvastatin impaired insulin signalling in adipose tissue from WT, but not IL-1β/- mice. Treatment with the caspase-1 inhibitor z-YVAD, prevented atorvastatin-inhibition of insulin signaling suggesting active IL-1b is required. The isoprenoid, Geranylgeranyl-pyrophosphate (GGPP, 50 µM), prevented atorvastatin-induced defects in insulin signalling. Interestingly, atorvastatin decreased insulin-stimulated lipogenesis in both WAT and 3T3-L1 adipocytes. Our data demonstrates that statin-induced reduction in isoprenoids that required for protein prenylation, impairs insulin action via an IL-1β mechanism in an adipose tissue.
Adipose derived protein, adipsin, regulates glucose and lipid uptake. by Joon Ho Moon | Yun Hye Kim | Ju Hee Lee | Jeongah Yoo | Joe Eun Son | Eashita Das | Florine Lenglin | Je Yuan Wang | James An | Sarah Kim | Weikang Cai | Chi-Chung Hui | Kyoung-Han Kim | C. Ronald Kahn | Hoon-Ki Sung | 1Translational Medicine Program, The Hospital for Sick Children | 1 | 1 | 1 | 2Developmental & Stem Cell Biology Program, The Hospital for Sick Children | 1 | 1 | 1 | 1 | 1 | 3Section of Integrative Physiology and Metabolism, Joslin Diabetes Center & Department of Medicine, Harvard Medical School | 2 | 2 | 3 | 1 & Dept Laboratory Medicine and Pathobiology, University of Toronto

Modern lifestyles favor longer periods of daily energy intake and shorter fasting period and these erratic eating patterns are associated with obesity and diabetes. Therefore, eating pattern regulations, including intermittent fasting (IF) have gained attention as a therapeutic strategy. White adipose tissue (WAT) exerts a significant impact on metabolism by producing adipokines. Erratic eating patterns are also related to dysregulation of adipokines. Thus, understand the metabolic functions of adipokines is important to pioneer treatments against metabolic disorders. Adipsin is an adipokine with protease activity and a member of the trypsin-family. Adipsin’s expression is elevated by fasting. Interestingly, adipsin levels are low in animal models of obesity and diabetes, suggesting the potential implication of adipsin in metabolism. However, adipsin’s metabolic function is unknown.

Our previous study showed that IF improves glucose homeostasis through expression of several adipokines. RNA-sequencing and serum analysis on IF-treated WAT revealed that adipsin was significantly increased. Interestingly, trypsin has insulin-like effect and induces phosphorylation of insulin receptor (IR) β-subunit by cleavage of the IR α-subunit, which inhibits autophosphorylation of IR β-subunit. In pilot analysis, I found significant similarities between trypsin with adipsin and IR with insulin-like growth factor 1 receptor (IGF1R), both in their amino acid sequences and 3D structure, suggesting that adipsin may phosphorylate IR or IGF1R through a cleavage of the α-subunit, like the trypsin. Therefore, I performed glucose and lipid uptake assay. My in vitro experiment demonstrated that adipsin directly increases glucose uptake through phosphorylation of Akt and translocation of glucose transport protein 4 (Glut4), and lipid uptake with unknown mechanism.

Our data suggest that adipsin have direct effects on glucose and lipid metabolism. Although the molecular mechanism of adipsin’s action is not fully understood, the novel glucose and lipid regulatory function of adipsin open a possibility of developing new therapeutic strategies for obesity.
Luman as a Novel Regulator of Lipid and Cholesterol Metabolism by Brandon Smith | Tiegh Taylor | Jenna Penney | Marica Bakovic | Ray Lu | University of Guelph | University of Guelph | University of Guelph | University of Guelph

Luman/CREB3 is an endoplasmic reticulum (ER)-transmembrane transcription factor considered part of the Unfolded Protein Response (UPR) which functions to alleviate ER stress and has been implicated in lipid metabolism. Luman KO mice have a lean phenotype and altered stress sensitivity. Luman Recruitment Factor (or CREBFRF), a regulator of Luman, plays a role in lipid metabolism. In addition, Luman/CREB3-like protein 3 (or CREB3L3), has a well-described role in the regulation of lipid and energy metabolism, through nuclear receptor protein-interactions and transcriptional regulation. CREB3L3-deficient mice show a lean phenotype similar to that of Luman KO mice. Luman-deficiency is also known to cause high glucocorticoid receptor (GR) expression which is another major regulator of metabolic genes. Preliminary studies show little to no weight gain, hepatic lipid accumulation, and altered plasma cholesterol levels in Luman KO mice on a HFD compared to wild-type. Expression of lipolytic and fatty acid oxidation genes are also elevated in liver tissue of Luman-deficient mice. This research could potentially lead to novel therapeutic targets for treating obesity, and obesity-related diseases such as diabetes, heart disease, stroke, or cancer. Taking all this together, we propose that Luman is a novel lipid and cholesterol metabolism co-regulator, which acts through functional interactions with various nuclear receptors.
n-3 PUFA induced n-6 PUFA bioconversion controls alpha-1-adrenoceptor expression in mice by Jiayu (Daisy) Ye | Sanjoy Ghosh | UBC-OKANAGAN | UBC-OKANAGAN

Excess n-6 PUFA like linoleic acid (LA) and low long chain n-3 PUFA like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as that seen in the Western world is now linked to an elevated risk of heart diseases. One mechanism that might be involved is the expression of vascular α1-adrenoceptors (α-1 Adr), important in regulating blood pressure, which is elevated with n-6 PUFA but lowered with n-3 PUFA diet. Although the underlying mechanism remains uncertain, we demonstrated recently that accumulation of LA is the cause for alteration of cardiac collagen leading to a ‘stiff’ heart. In this study, we hypothesized that n-3 PUFA facilitates the clearance of LA and inhibits α1-Adr expression and inflammatory signalling in the vasculature of n-6 PUFA fed mice. We utilized mice with defective PUFA bioconversion capacity in lacking elongase 5 (ELOVL5+/−) to study the impact LA accumulation under isocaloric 40% high fat diets rich in corn (LA rich), corn + fish oil (n-3 PUFA rich) and olive oil (control; monounsaturated fatty acid rich). Using gas chromatographic analysis of aorta lipids, we demonstrate that accumulation of upstream LA and gamma linoleic acid (GLA) independent of ARA, increases α1-Adr expression in the aorta under corn oil diet but not olive oil diets. This accumulation is prevented by fish oil supplementation which facilitated ELOVL5 regulated LA/GLA bioconversion and clearance to longer chain PUFA. We believe this novel metabolic function of n-3 PUFA in accelerating n-6 PUFA bioconversion via ELOVL5 remains a novel mechanism in the anti-inflammatory and cardioprotective action of long chain n-3 PUFA, in the context of a high n-6 PUFA diet in the Western world.
Crosstalk Between the Kidneys and Pancreas in Glucose Control by Maria Fernanda Fernandes | Iman M‘Hiri | Phillip Marvyn | Robin E. Duncan | University of Waterloo | University of Waterloo | University of Waterloo

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**Background:** Impaired renal function is a risk factor for new onset diabetes, although the mechanism(s) involved are not well understood. Renal triacylglycerol (TAG) lipid droplet accumulation (renal steatosis) develops in, and is worsened by, increasing obesity, which is a risk factor for both kidney disease and diabetes. Lipid droplets also accumulate in the kidneys of diabetics. This suggests that renal steatosis might cause kidney damage, which could then lead to diabetes. To test this hypothesis, we have generated a mild, kidney-specific steatosis model by targeted ablation of a triglyceride lipase in renal tubules (RT-SAKO mice). **Objectives:** 1) To determine if RT-SAKO mice develop impaired glucose metabolism; 2) To determine if impaired glucose metabolism occurs prior to, or after the development of kidney steatosis (i.e. the cause-and-effect relationship between kidney fat changes and impaired glucose control). **Results:** Intraperitoneal glucose tolerance testing indicated significantly impaired glucose disposal, but no significant impairment in insulin-mediated glucose disposal by 16-17 weeks of age. Both glucose and insulin tolerance were normal at 9-10 weeks. Euglycemic-hyperinsulinemic clamps performed (in collaboration with Dr. Andre Marette, Universite de Laval) also showed no difference on the glucose-infusion rate required to maintained euglycemia during the insulin infusion, indicating similar levels of systemic insulin sensitivity between RT-SAKOs and littermate controls. Interestingly, no evidence of kidney inflammation or fibrosis was evident. **Conclusions:** More than half a billion people globally have, or are at risk for diabetes as a result of kidney disease. Understanding how problems in the kidney cause diabetes could help prevent and treat both diseases.
Phosphorylation of serine 315 and 319 is inversely related to activation of CTP:phosphocholine cytidylyltransferase alpha

by Michael J. McPhee | Neale D. Ridgway | Stephen Pelech | Department of Biochemistry & Molecular Biology, Dalhousie University, Halifax, NS | Department of Biochemistry & Molecular Biology, Department of Pediatrics, Dalhousie University, Halifax, NS | Department of Medicine, University of British Columbia and Kinexus Bioinformatics, Vancouver, BC

The nuclear enzyme CTP:phosphocholine cytidylyltransferase alpha (CCTα) catalyzes the rate-limiting step of the CDP-choline pathway for biosynthesis of phosphatidylcholine (PC) in mammalian cells. Domain M of CCTα is an amphipathic helix that embeds into membranes enriched in anionic lipids and or depleted of PC, leading to enzyme translocation and activation at the nuclear envelope (NE). Adjacent to domain M is the P-domain, which contains 16 serine and threonine residues that are phosphorylated and negatively regulate CCTα activation. However, the P-domain phosphorylation sites and relevant kinases/phosphatases involved in regulation of CCTα membrane association and activity are poorly understood. In this study, we used phospho-specific antibodies against Ser315/Ser319 and Tyr359/Ser362 in the P-domain to investigate how phosphorylation regulates CCTα activity in IEC-ras and HeLa cells. CCTα activation with oleate/BSA induced dephosphorylation of Ser315/Ser319 but not Tyr359/Ser362 in IEC-ras and HeLa cells. Similarly, choline depletion-induced CCTα activation also caused dephosphorylation of Ser315/Ser319 but not Tyr359/Ser362. Confocal microscopy revealed translocation of CCTα to the NE when activated by oleate/BSA or choline depletion but membrane-associated CCTα was not phosphorylated on Ser315/Ser319. Our data suggest that phosphorylation of Tyr359/Ser362 was unaffected by enzyme activation and may not contribute to enzyme regulation under these conditions. In contrast, dephosphorylation of Ser315/Ser319 was associated with enzyme activation at the NE. These phospho-specific antibodies will be useful reagents to further investigate phospho-regulation of CCTα activity in pathological contexts that affect enzyme activity.
Lack of endonuclease G reduces linoleic acid accumulation and protects the cardiac muscle from inflammation. by Jiayu (Daisy) Ye | SANJOY GHOSH | UBC-OKANAGAN | UBC-OKANAGAN

Endonuclease G (EndoG) is a mitochondrial endonuclease associated with cardiac apoptotic pathway. While the actions of endonucleases are commonly attributed to DNA changes, links to lipid metabolism remain unanswered. In a previous study, inhibition of EndoG rescues endothelial cells from modified LDL mediated toxicity but its impact on lipid pathways were not studied. In this study, we utilized EndoG heterozygous knockout (+/-) mice on a C57 background (with low circulating cholesterol) to evaluate the role of this endonuclease on cardiac muscle metabolism. EndoG +/+ and +/- mice were fed corn oil (40% energy from fats, rich in n-6 polyunsaturated fatty acids) for 6 weeks. Gas chromatography (GC) analysis of the heart showed that in EndoG +/- mice, there was a surprising increase in the conversion of upstream n-6 PUFAs like linoleic acid (LA) to downstream PUFAs. Such increased clearance of LA was also associated with a protective phenotype as demonstrated by a downregulation of cardiac collagen production, a reduction in cytokines like interferon gamma, TNF-alpha as well as upregulation of DNA repair gene expression in EndoG +/- heart muscle and serum. In vitro, fibroblasts were transfected with endoG Orf or empty vector, and exposed to various fatty acids. These experiments also showed that upregulation of EndoG increased linoleic acid (LA) accumulation and decreased its bioconversion to other downstream PUFAs. In summary, this study demonstrates a novel role of EndoG in regulating bioconversion of PUFAs in the cardiac muscle.

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Functional studies of ABC sterol transporters in lipid-bilayer nanodiscs by William Jennings | Bala M. Xavier | Aiman Zein | Jyh-Yeuan Lee | Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON, CA | Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON, CA | Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON, CA | Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON, CA

Abnormal elevation of plasma cholesterol is a key biomarker for developing atherosclerosis and is among the top risk factors for cardiovascular diseases. Cholesterol is obtained from the diet or by de novo biosynthesis from acetyl-CoA. To maintain cholesterol balance, circulating high-density lipoprotein and low-density lipoprotein particles serve as conduits for the transport of cholesterol and other lipids between tissues, whereas excess cholesterol and dietary sterols are excreted by either chemically converting cholesterol to bile acids or by secreting the sterols into the bile in the liver. The ATP-binding cassette (ABC) transporters play a critical role in facilitating cholesterol efflux across cellular membranes and their deficiency is associated with hypercholesterolemia. Using the subfamily-G ATP-binding cassette (ABCG) sterol transporters, this project aims to identify the determinants of catalytic activity and transport-competence of the active sterol transporters in the native membrane environment. First, we have developed a nanodisc reconstitution protocol for the well-characterized sterol transporter ABCG5/G8. Second, we are developing a detergent-free purification of the sterol transporters to isolate recombinant proteins in a native lipid-bound state. We present here a method to reconstitute active ABCG5/G8 in lipid nanodiscs and the preliminary solubilization analysis of ABCG5/G8 using styrene-maleic acid (SMA) copolymers. Our future directions include: 1) to establish the catalytic profile of reconstituted ABCG5/G8, 2) to determine the functional oligomers of ABCG5/G8 in lipid bilayers, and 3) to facilitate structural studies using single-particle cryo-electron microscopy (cryo-EM). The major goal is to elucidate the molecular events underlying the transport mechanism of the ABC cholesterol transporters, providing a platform for pharmacological discovery to modulate sterol transport activity in cardiovascular and metabolic diseases.
Intermittent Fasting Improves Age-Associated Metabolic Abnormalities by Rejuvenation of White Adipose Tissue

Hee Lee, Eashita Das, Yun Hye Kim, Joanna Yeung, Yanqing Jiang, Min-Ah Choi, Jae-Ryong Kim, Hoon-Ki Sung | Translational Medicine, The Hospital for Sick Children, Toronto, Canada

Background: Adipose tissue aging is a hallmark of age-associated metabolic dysfunctions. In particular, recent studies demonstrate that adipose progenitor cells (APCs) adopt a fibrogenic phenotype to drive a pathological phenotype. We previously demonstrated that intermittent fasting (IF) protects mice from diet-induced obesity and improves metabolic health by browning of the white adipose tissue (WAT). However, as chronic inflammation and fibrosis is established in WAT by age and extended high-fat diet, it is unknown whether IF will retain its benefits in age-associated metabolic abnormalities.

Methods: We performed 6 weeks of 2:1 intermittent fasting (2 days of feeding - 1 day of fasting) in a mouse model that displays diet- and age-induced metabolic abnormalities (aged DIO), such as high body weight, insulin resistance, and fatty liver.

Results: Mice subjected to short-term IF exhibited significantly reduced body weight, fat mass, improved glucose tolerance, insulin sensitivity, and substantial lipid clearance in the liver. Although IF-treated aged DIO did not display WAT browning, age- and diet-associated fibrotic progression was significantly repressed in WAT. Interestingly, RNA sequencing data revealed that aging-associated pathways were significantly suppressed in WAT. In particular, APC-specific aging marker (i.e. CD9) was significantly reduced in IF-treated WAT, suggesting that IF may increase the stemness of APCs in aged WAT.

Conclusions: Age-induced impairment in adipogenic and angiogenic potential of adipose tissue can contribute to systemic inflammation and metabolic alterations, such as insulin resistance. Here, we demonstrate that IF improves metabolic health by rejuvenation of the white adipose tissue. Indeed, IF may be a viable treatment option to combat age-associated metabolic dysfunctions.
VEGF-A pathway regulates trans-endothelial lipid uptake by Yun Hye Kim, Joe Eun Son, Hira Raheel, Ju Hee Lee, Eashita Das, Jae-Ryong Kim, Jin Gyoon Park, Chi-chung Hui, Andras Nagy, Philhan Kim, Warren Lee, So Young Park, Kyung-Oh Doh, Hoon-Ki Sung | The Hospital for Sick Children

Obesity and increased fat intake have been widely associated with ectopic lipid accumulation in non-adipose tissues, resulting in various metabolic diseases. Thus, a better understanding of lipid uptake and tissue distribution is required to develop novel therapeutic strategies to treat abnormal lipid deposition and adverse metabolic consequences. However, little is known about the transport mechanism of dietary lipid from circulation to peripheral tissues. The endothelial cells regulate the communication between blood and peripheral tissues by regulating the transportation of essential nutrients and bioactive molecules. Recent studies have shown that vascular endothelial growth factor (VEGF) signal may regulate endothelial lipid uptake through increased membrane translocation of fatty acid transporting proteins. Similarly, our previous study also suggests a potential role of VEGF-A in trans-endothelial lipid transport, as we found elevated serum FFA levels in adipose-specific VEGF-A knock out (VEGF\textsuperscript{AdKO}) mice. In this study, we also confirmed that serum FFA levels are significantly decreased in adipose-specific VEGF overexpression (VEGF\textsuperscript{AdTg}) mice. Additionally, VEGF treatment increased FFA uptake in cultured endothelial cell, and this effect was abolished by VEGF-A antibody treatment. Taken together, this study suggests that VEGF-A pathway might be implicated in trans-endothelial lipid uptake, thereby regulating the balance of FA concentration between plasma and tissue.
Renal Steatosis Causes Onset of Glucose Intolerance In the Absence of Kidney Inflammation by Iman M’Hiri | Maria Fernanada Fernandes | Phillip M.

Marvin | Robin E. Duncan | Department of Kinesiology, University of Waterloo | Department of Kinesiology, University of Waterloo | University of Waterloo, McMaster University | Department of Kinesiology, University of Waterloo

Advancing age is a risk factor for both chronic kidney disease (CKD) and type II diabetes mellitus (T2DM). While hyperglycemia is well established as an initiator for CKD, it was recently discovered that kidney damage can reciprocally initiate T2DM, indicating a vicious cycle. Mechanisms mediating this relationship are poorly understood, however, a common characteristic of both conditions is the presence of ectopic fat in the kidneys. To isolate the direct effects of renal steatosis on the dysregulation of glucose metabolism, we have developed non-obese mice that have elevated renal triacylglycerol stores (RT-SAKOs), mimicking the ectopic lipid storage characteristic of patients suffering from CKD. RT-SAKO mice become glucose intolerant at 16 weeks-of-age, but have normal glucose tolerance at 10 weeks-of-age. To understand the progression and impact of renal steatosis on dysregulated glucose control, studies were conducted on mice prior to (i.e. 9-11 weeks old), during (i.e. 16-18 weeks old), and post (i.e. 23-25 weeks old) onset of glucose intolerance. Kidney gene expression of the inflammatory markers IL-6, IL-1B, Fibronectin, and TNFα was analyzed, and no significant differences were found between RT-SAKO and control mice at any time points. Our findings demonstrate that renal steatosis-induced hyperglycemia occurs in the absence of kidney inflammation, suggesting other factors play a role in the phenotype. Further studies will improve our understanding of renal steatosis and blood glucose regulation, with implications for the early detection of renal-metabolic disease.
Protective effects of phyto-oxylipin falcarinol in intestinal and systemic inflammation by Amanda Stefanson | Marica Bakovic | University of Guelph

Intestinal inflammation and problems with barrier integrity contribute to many intestinal diseases (IBD, IBS, celiac disease) but also have been implicated in other widely divergent pathologies (autoimmune diseases, food allergies, obesity, endotoxemia, chronic inflammation and even damage from intense exercise). Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a transcription factor that regulates the expression of a battery of antioxidant, anti-inflammatory and cytoprotective enzymes including heme oxygenase-1 (Ho-1). We evaluated the protective effect of the phyto-oxylipin falcarinol (FA) as a pre-treatment against lipopolysaccharide (LPS)-induced intestinal and systemic inflammation in mice in comparison to sulforaphane (SF), which is recognized as the most potent natural product activator of Nrf2. Phytochemical pretreatment effectively reduced the magnitude of intestinal pro-inflammatory gene expression with FA showing more potency than SF. FA was also more effective in upregulating Ho-1 at mRNA and protein levels in both mouse liver and intestine. Ho-1 was further upregulated at the protein level in the intestine only in FA during initial resolution, whereas in the liver, Ho1 protein was upregulated only in SF. FA but not SF attenuated plasma chemokine eotaxin and white blood cell growth factor GM-CSF, which are involved in the recruitment and stabilization of first-responder immune cells. During initial resolution, only FA increased M2/Th2-type cytokines (IL-4, IL-13, IL-10) as well as cytokines associated with Th9 cell expansion (IL-2, IL-9). Both phytochemical pretreatments protected against LPS-induced reduction in intestinal barrier integrity, but FA additionally reduced inflammatory cell infiltration even below negative control.
Location
60 Leonard Avenue - Krembil Discovery Tower | Toronto, ON M5T 0S8.

When you arrive at the BMO Education & Conference Centre, you will enter through the main doors of the Krembil Discovery Tower (60 Leonard Avenue; south west corner of Leonard / Nassau). Upon entry through the main doors, the Centre is located to the left.

If you enter through Toronto Western Hospital, please follow the signs that state “KREMBIL” as this will direct you into the Krembil Discovery Tower where the BMO Education & Conference Centre is located.

Parking is available at the BMO Education & Conference Centre via two lots off of Nassau Street. The first is located at the corner of Bathurst and Nassau and the second lot entrance is located on Nassau Street just east of Leonard Avenue. The parking lots remain open; provided they are not at capacity. All standard payment methods are accepted. For current rates, please call the parking office at 416-214-1339. Wheelchair-accessible parking is available at all UHN parking lots.

Green P Parking / Toronto Western Hospital
35 Bellevue Avenue – 240 meters from BMO Conference & Education Centre
20 St. Andrew Street (Kensington Garage) – 700 meters from BMO Conference & Education Centre
201 Claremont Street – 950 meters from BMO Conference & Education Centre

Public Transit – Streetcar
- 511 Bathurst St & Nassau St Stop o Walk east on Nassau to the BMO Centre
- 505 Dundas St West & Bathurst St o Walk through the hospital, following the KREMBIL signs to the BMO Centre or proceed north on Bathurst St to Nassau St and then east on Nassau St to the BMO Centre
- 506 College St & Bathurst St o Get off at Bathurst St and walk south down Bathurst St to Nassau St, proceed east on Nassau St to the BMO Centre

SHUTTLE: There is a free UHN shuttle service that runs Monday through Friday, between Toronto Western Hospital and Toronto General Hospital. The shuttle leaves Toronto General Hospital (University Avenue entrance) 6:15am this is the first shuttle in the morning, it runs until 8:15pm.

When you arrive at Toronto Western Hospital on the shuttle, you walk north on Leonard Avenue to access the main entrance to the Krembil Discovery Tower where the BMO Education & Conference Centre is located (south west corner of Leonard / Nassau).

If you’re coming from TWH, please use the entrance via Second Cup; follow the hallway around to the Main Entrance of the Krembil Discovery Tower.
Directions to the Tall Ship Kajama Awards Banquet

Saturday, June 9th

Boarding: 6:30 to 7:00 PM

Harbourfront & Toronto Islands Sailing, Dinner & Awards: 7:00 to 11:00 PM

Return to Dock: 11:00 PM
The parking revenues help to finance public programming and activities at Harbourfront Centre which is a non-profit charitable organization.

Parking Information: 416-973-4875