CLVS

Canadian Lipid & Vascular Summit

PROGRAM

September 25 – 28, 2025

Delta Hotels Bessborough
Saskatoon, SK, CANADA

Delta Hotels Bessborough
601 Spadina Crescent E
Saskatoon, SK S7K 4H9

September 25- 28, 2025

Thursday, Sept 25th	**2-5pm**	**Arrival and Registration**
	5:00-6:30pm	Reception with food
	6:30-6:45pm	**Welcome and Opening Remarks**
	6:45-8:30pm	**Session 1: Atherosclerosis and Diabetes** **Chairs: Sebastien Gauvrit, Scott Widenmaier**
	6:45-7:15pm	***Altschul Lecture*** **Dr. Gordon Francis** University of British Columbia *Smooth Muscle Cell Foam Cells: Differences from Macrophage Foam Cells and Their Importance as a Specific Therapeutic Target*
	7:15-8:30pm	**Oral abstract presentations** (10min + 5min Q)
		O-1 Xiaobei Li PhD Student (Université de Montréal) *Saturated and Unsaturated Fat–Based Ketogenic Diets Attenuate Atherosclerosis in Atheroprone Male Mice*
		O-2 Rida Khan Masters Student (University of British Columbia) *Investigating early single-cell changes to unravel the chronology of cardiovascular complications in type 1 diabetes*
		O-3 Khushali Trivedi PhD Student (University of Manitoba) *Sirtuin 3 Deficiency in the Liver is Associated with Mitochondrial Dysfunction and Hepatic Steatosis in Gestational Diabetes*
		O-4 Samuel Leung Masters Student (University of British Columbia) *Spatial Gene Expression of Human Coronary Arteries Revealed the Molecular Features of Diffuse Intimal Thickening in Explanted Hearts*
		O-5 Andria Henry PhD Student (University of Toronto) *Lipoprotein(a) and the Arterial Endothelium: Elucidating Mechanisms of Residual Cardiovascular Risk*
	8:30pm-	Networking activities

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Friday, Sept 26th	7:30-8:30am	Breakfast
	8:30-10:00am	**Session 2: Heart and Liver Health** **Chairs: Erin Mulvihill, Vernon Dolinsky**
	8:30-9:15am	***Dr. Gary Lopaschuk Memorial Lecture*** **Dr. John Ussher** University of Alberta *The Regulation of Cardiac Fatty Acid Metabolism via Malonyl CoA in Health and Disease*
	9:15-10:00am	**Oral abstract presentations** (10min + 5min Q)
		O-6 Jeong-Ah Yoo PhD Student (McMaster University) *ApoA1 and HDL Selectively Protect the Heart During Doxorubicin Chemotherapy via Hepatic SR-B1*
		O-7 Aaron Getachew Masters Student (University of Alberta) *Hepatocyte Prosaposin Deficiency Alters Fibrotic Remodeling in a Sex-Specific Manner during Metabolic Dysfunction-Associated Steatohepatitis (MASH) Progression*
		O-8 Afroza Ferdouse PhD Student (University of Alberta) *Deciphering the role of retinoic acid signaling in alcohol-associated liver disease using AlbCre:RARdn mice*
	10:00-10:15am	Coffee Break
	10:15-12:00pm	**Session 3: Regulators of Lipid Metabolism** **Chairs: Scot Stone, Robert Brown**
	10:15-11:00am	***Rubenstein lecture*** **Dr. Stephen G Young** University of California Los Angeles *New Insights into APOA5 Biology*
	11:00-12:15pm	**Oral abstract presentations** (10min + 5min Q)
		O-9 Jihong Lian Research Associate (University of Alberta) *Arylacetamide Deacetylase (AADAC) Regulates Hepatic Lipid Hydrolysis and De Novo Lipid Synthesis*
		O-10 Derek W. Stouth Postdoctoral Fellow (McMaster University) *Targeting IP3R1, the major ER calcium release channel in the liver, as a means of lowering PCSK9 expression and secretion*
		O-11 Jianfan Ivy Nie Masters Student (University of Ottawa) *Disrupted AMPK-mTORC1 Signaling Alters Liver Metabolism in Mice*
		O-12 Shabnam Pourshojae Masters Student (University of Ottawa) *Understanding the role of Irx1 and Irx2 transcription factors in pancreatic alpha cell identity*

		O-13 Julien Wourms Masters Student (University of Alberta) *The Vagus Nerve Mediates NTS Glucagon Signalling to Lower Triglyceride-Rich Very Low-Density Lipoprotein Secretion*
	12:15-1:15pm	Lunch
	1:15-3:15pm	**Session 4: Stewart Whitman and Jean Davignon Lectures** **Chairs: Morgan Fullerton, Changting Xiao**
	1:15-2:00pm	***Stewart Whitman Lecture*** **Dr. Emily Day** Western University *Mitochondrial Stress Meets Immunity: The Role of the mtUPR and Mitochondrial Proteases in Macrophage Inflammation*
	2:00-2:45pm	***Jean Davignon Lecture*** **Dr. Khosrow Adeli** University of Toronto *Postprandial Lipid Metabolism: Mechanistic Pathways from Experimental Models to Human Physiology*
	2:45-3:15pm	**Group photo**
	3:15-6:00pm	**Trainee Networking** No sign-up required!
	6:00pm	Dinner on your own
	7:00-8:00pm 8:00-9:00pm	CSATVB Exec/Directors Meeting CLC Business Meeting

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Saturday, Sept 27th	7:30-8:30am	Breakfast
	8:30-10am	**Session 5: CSATVB – KSoLA Joint Symposium on Dyslipidemia** **Chairs: Warren Lee, Bernardo Trigatti**
	8:30-9:15am	***Physician Scientist Lecture*** **Dr. Daniel Gaudet** Université de Montréal *Meeting the Challenges of Tomorrow in the Management of Severe and Extreme Hypertriglyceridemia*
	9:15-9:45am	**Dr. Sang Min Park** Division of Cardiology, Nowon Eulji Medical Center *Clinical Benefits of Early Statin and Ezetimibe Combination Therapy for Primary Prevention in People with Dyslipidemia*
	9:45-10:30am	**Oral abstract presentations** (10min + 5min Q)
		O-14 Reihane Taheri PhD Student (University of Alberta) *The association of healthy eating index score and n-3 fatty acid intake with cardiovascular diseases incidence and lipid biomarkers in Alberta's Tomorrow Project cohort*
		O-15 Hannah Zhang PhD Student (University of Manitoba) *Lysophosphatidylcholines as early markers and molecular mediators of AMI-induced cardiogenic shock*
		O-16 Elad Shemesh Postdoctoral Fellow (McMaster University) *Extreme HDL and risk for cardiovascular disease and mortality: An ancestry-based analysis*
	10:30-10:45am	Coffee Break
	10:45-12:15pm	**Session 6: Immunometabolism** **Chairs: Rami Al Batran, Scott Widenmaier**
	10:45-11:30am	**Simon Pierre Noel Lecture** **Dr. Katey Rayner** University of Ottawa Heart Institute *Cross-talk of Lipids & Inflammation in Cardiometabolic Disease*
	11:30-12:15pm	**Oral abstract presentations (10min + 5min Q)**
		O-17 Ruoqi Yu Masters Student (University of Saskatchewan) *Oxidized phosphatidylcholine induced neurodegeneration in the grey matter is partially dependent on necroptosis activation and is mitigated by microglia*
		O-18 Ali A. Abdalbari Masters Student (University of Ottawa) *Dipeptidyl Peptidase-4 (DPP4) at the Crossroads of Metabolism, Inflammation, and Atherosclerosis*
		O-19 Anthony Parent PhD Student (McGill University)

		Targeting PRMT5 limits plaque growth by blunting macrophage proliferation and boosting efferocytosis
	12:15-1:30pm	Lunch
	12:15-1:30pm	CSATVB Annual General Meeting
	1:30-3:00pm	**Session 7: Vascular Biology and Emerging Therapies** **Chairs: Jessica Yue, Scott Widenmaier**
	1:30-2:00pm	**Dr. Sebastien Gauvrit** University of Saskatchewan *Cellular and Molecular Dynamics of Vascular Development*
	2:00-3:15pm	**Oral abstract presentations (10min + 5min Q)**
		O-20 Christopher Yuen PhD Student (University of British Columbia) *Early hyperactivation of endothelial-specific pathways prevents diabetes-associated chronic vascular damage*
		O-21 Tianyu Hang Postdoctoral Fellow (University of Saskatchewan) *Characterization of Gut-Derived Extracellular Vesicles during Active Lipid Absorption*
		O-22 Umar Farouk Mustapha Postdoctoral Fellow (University of Saskatchewan) *M30, a Thirty Amino Acid Mid-Segment of Nesfatin-1 Exhibits Potential Lipid Lowering Effects in a Human Liver Cell Line*
		O-23 Sora Kwon Postdoctoral Fellow (University of Ottawa) *Empagliflozin Ameliorates Hepatic Steatosis and Fibrosis via Ketogenesis-Dependent and -Independent Mechanisms*
		O-24 Farnoosh Tabatabaeian Masters Student (University of Saskatchewan) *GLP-1R/GIPR Dual Agonism Reduces Intestinal Lipid Secretion*
	3:30-5:30pm	**Poster session**
	6:00pm	**Dinner + Trainee Award Presentation + Recreation**
Sunday, Sept 28th	**Departure**	

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ABSTRACT BOOKLET

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Oral Presentations

O-1

Saturated and Unsaturated Fat–Based Ketogenic Diets Attenuate Atherosclerosis in Atheroprone Male Mice

Xiaobei Li[1,2,3], Abdualrahman Mohammed Abdualkader[1,2,3], Elizabeth Lacroix[4], Jiaheng Guan[1,2,3], Yiming Yin[1,2,3], Catherine Martel[4], Rami Al Batran[1,2,3]

[1] Faculty of Pharmacy, Université de Montréal
[2] Montreal Diabetes Research Center
[3] Cardiometabolic Health, Diabetes and Obesity Research Network
[4] Montreal Heart Institute, Montréal, QC, Canada

Background: Ketogenic diets (KDs), characterized by very low carbohydrate and high fat intake, are increasingly popular for weight loss. Classical KDs are typically high in saturated fats, raising concerns that long-term adherence may promote atherosclerosis. Evidence from other dietary patterns suggests that replacing saturated with unsaturated fats can slow atherosclerosis progression. However, the influence of fat composition within KDs on cardiovascular risk and weight regulation remains unclear.
Hypothesis: We postulated that a saturated fat–based KD (SF-KD) would accelerate atherosclerosis, whereas an unsaturated fat–based KD (UF-KD) would not, with both diets producing comparable weight loss.
Methods: Male LDL receptor–deficient mice were made obese with a high-fat diet (HFD) and then randomized to 18 weeks of HFD, SF-KD, or UF-KD, each supplemented with cholesterol. A low-fat diet (LFD) group served as a healthy control.
Results: Compared with the HFD group, mice fed SF-KD and UF-KD exhibited markedly smaller lesions in the aortic sinus, with reductions of approximately 57% and 62%, respectively. Nevertheless, lesion sizes in both KD groups remained nearly twice as large as those in the LFD group. Quantitative analysis revealed that necrotic core area, collagen content, and VCAM-1 positive staining were significantly reduced in both KD groups relative to HFD, reaching values comparable to LFD. By contrast, α-SMA positive area did not differ between HFD and KD groups, and both were significantly higher than in the LFD group. Plasma cholesterol in the VLDL and LDL fractions was significantly lower in KD-fed mice than in HFD-fed mice, although still higher than in the LFD group. Inflammatory markers (TNF-α, IL-6, VCAM-1, ICAM-1, MCP-1) were strongly elevated in HFD-fed mice but returned to levels comparable to LFD in both KD groups. Consistent with this, KEGG pathway analysis showed that both KDs downregulated several inflammatory pathways, including NOD-like receptor signaling and cytokine–cytokine receptor interaction. Macrophage coverage within lesions ($CD45^+CD68^+$) was comparable across groups. However, both KDs significantly increased the M2/M1 macrophage polarization ratio compared with the HFD group, indicating a shift toward an anti-inflammatory environment. Ex vivo assays further showed that both KDs, particularly SF-KD, markedly reduced ox-LDL uptake relative to HFD, which in turn limited foamy cell formation during the early stage of lesion development. Both KD interventions induced rapid weight loss after the dietary switch. However, mice in the UF-KD group began to regain weight after two weeks. By the end of the study, their body weight was close to that of the HFD group. In contrast, SF-KD mice maintained lower body weight throughout, remaining only slightly above LFD levels.
Conclusions: In LDL receptor–deficient mice, both saturated and unsaturated fat–based KDs reduced systemic inflammation and atherosclerotic plaque burden compared with an obesogenic HFD, yet both remained atherogenic relative to a healthy LFD. UF-KD conferred no advantage over SF-KD in limiting atherosclerosis, whereas SF-KD was more effective in sustaining weight loss.

PhD Student

O-2

Investigating early single-cell changes to unravel the chronology of cardiovascular complications in type 1 diabetes

Rida Khan[1,3], Taylor A. Ricci[1,3], Puja Biswas[1,3], Constadina Panagiotopoulos[1,3], Pascal Bernatchez[2], Angela M. Devlin[1,3]

[1] Department of Pediatrics, The University of British Columbia, Vancouver, Canada; [2] Department of Anesthesiology, Pharmacology and Therapeutics, The University of British Columbia, Vancouver, Canada; [3] BC Children's Hospital Research Institute, Vancouver, Canada

Introduction: Type 1 diabetes (T1D) is an autoimmune disease marked by the destruction of insulin-producing pancreatic beta cells and is the predominant form of diabetes diagnosed in childhood. T1D-associated hyperglycemia causes vascular damage and afflicted individuals have a 10-fold higher risk of early-onset cardiovascular disease. However, the chronology and pathophysiology of vascular damage, especially in the early phase, is not fully understood. This study aims to investigate molecular mechanisms and the chronology of vascular damage during pediatric diabetes progression using a mouse model to identify biomarkers of early vascular damage.
Hypothesis: We hypothesize that sex-specific cardiovascular damage is already present at diabetes onset and worsens over time in pediatric T1D.
Methods and Results: Single-cell RNA sequencing (scRNA-seq) was conducted on whole aorta from male and female C57BL/6J Ins2+/Akita mice, a model of pediatric diabetes, and age-matched control littermates at: 1) diabetes onset (blood glucose ≥16.7mmol/L); and 2) 4 weeks post-diabetes onset. Male and female Ins2+/Akita mice develop diabetes at ~age 4 weeks and 6 weeks, respectively. At diabetes onset, Ins2+/Akita male aorta exhibited lower T-cell populations, more vascular smooth muscle cells (VSMC) and fibroblasts along with upregulated inflammatory pathway gene expression in endothelial cells (ECs) compared to age-matched controls. At 4 weeks post-onset, Ins2+/Akita male aorta shifts towards a greater immune cell population and reduced VSMC and fibroblasts compared to controls. At both time points, upregulation of pathways related to vascular development was observed in ECs from Ins2+/Akita male aorta. Female Ins2+/Akita aorta at diabetes onset and post-onset had reduced fibroblast and VSMC populations and showed greater upregulation of inflammatory pathways in ECs at post-onset of diabetes compared to controls. Analysis of gene expression identified upregulation of pathways related to muscle differentiation and extracellular matrix organization in male and female Ins2+/Akita aorta at both timepoints. Immunohistochemical analysis is underway to validate the presence of T-cell subtypes, assess vascular remodelling, and confirm the expression of relevant differentially expressed genes derived from scRNA-seq data. Intima media-thickness of the aortic arch is being measured to quantify early structural changes in vascular tissue during the progression of diabetes.
Conclusions: The current identification of sex-specific cellular and gene expression changes, including early immune activation at diabetes onset in a mouse model of pediatric diabetes, supports the concept that major vascular damage may already be present in the pre-clinical phases of the disease, which will likely contribute to the initiation and progression of vascular complications in diabetes. These findings will help uncover biomarkers of early vascular damage that guide the development of targeted interventions to prevent and treat cardiovascular complications in children with diabetes.

Masters Student

O-3

Sirtuin 3 Deficiency in the Liver is Associated with Mitochondrial Dysfunction and Hepatic Steatosis in Gestational Diabetes

Khushali Trivedi[1,3,4], Bo Xiang[1,3,4], Jewel Paskaruk[1,3,4], Ayesha Saleem[2,3,4], Vernon W. Dolinsky[1,3,4]

[1] Department of Pharmacology and Therapeutics, University of Manitoba; [2] Department of Oral Biology, University of Manitoba; [3] Children's Hospital Research Institute of Manitoba; [4] Diabetes Research Envisioned & Accomplished in Manitoba, Winnipeg, MB

Background: Pregnancy is characterized by profound metabolic changes in maternal metabolism that serve to support the growth and development of the fetus. Gestational diabetes mellitus (GDM) is the most common transient metabolic disorder during pregnancy. GDM significantly increases the post-pregnancy risk of type 2 diabetes and obesity. GDM is characterized by insulin resistance and glucose intolerance, though the molecular mechanisms responsible remain incompletely understood. Previously we identified hepatic lipid accumulation during pregnancy as a central feature driving impaired glucose homeostasis. Sirtuin-3 (SIRT3) is a mitochondrial NAD^+-dependent deacetylase that regulates oxidative metabolism, including fatty acid beta-oxidation, particularly in the liver, though its function during pregnancy has not been investigated. Recently, we discovered that hepatic SIRT3 expression was reduced in rodent models of GDM.
Hypothesis: Liver-specific deficiency of SIRT3 impairs mitochondrial fatty acid oxidation, leading to hepatic steatosis, dyslipidemia, and glucose intolerance during pregnancy, that are characteristic of GDM.
Methods: Liver-specific SIRT3 knockout mice (SIRT3-LKO) were generated by crossing Sirt3tm1.1Auwmice from Jackson Labs with loxP sites flanking exons 2-3 of the Sirt3 gene with albumin-promoter driven cre-recombinase mice. SIRT3-LKO mice and Cre-negative controls were fed either a low-fat diet (10% kilocalorie fat) or high-fat-sucrose diet (45% kilocalorie fat) for 6-weeks before pregnancy and throughout the 3-week mouse pregnancy to induce GDM. Glucose tolerance tests were performed at gestational day (GD16). At GD18, maternal serum and liver tissues were collected. Hepatic steatosis was visualized by hematoxylin and eosin and quantified using Oil Red O staining. Liver and serum triglycerides and circulating free fatty acids were quantified. Mitochondrial function was assessed using the Agilent Seahorse XFe24 Analyzer to measure complex I- and fatty acid–driven respiration in isolated liver mitochondria.
Results: Pregnant SIRT3-LKO mice exhibited glucose intolerance (1.3-fold, $p<0.01$) and serum insulin levels elevated by 1.5-fold ($p<0.01$) compared to controls. Histological analysis of the liver showed marked hepatic steatosis, with Oil Red O–positive area increased by 2-fold ($p<0.0001$) in SIRT3-LKO mice only during pregnancy. Biochemical analysis confirmed 1.8-fold elevation in hepatic triglyceride levels ($p<0.05$), increased serum triglycerides (1.4-fold, $p<0.01$) and 2-fold elevation in circulating FFAs ($p<0.05$). Mitochondrial respiration was significantly impaired, with fatty acid oxidation reduced by 2.5-fold ($p<0.001$) and complex I–linked basal respiration reduced by 1.4-fold ($p<0.0001$) in SIRT3-LKO liver mitochondria.
Conclusions: SIRT3 is a key regulator of hepatic mitochondrial function and lipid metabolism during pregnancy at a stage when maternal demands for energy production are high. Liver-specific SIRT3 deficiency disrupts fatty acid oxidation and promotes lipid accumulation in the liver, leading to elevated circulating lipids, hyperinsulinemia, and glucose intolerance—metabolic hallmarks of GDM. These findings provide mechanistic insights that link mitochondrial dysfunction during pregnancy to hepatic steatosis and impaired control of blood glucose levels during pregnancy, key features in the pathogenesis of GDM. Future studies will investigate whether activation of SIRT3 can reverse hepatic steatosis and improve glycemic control during pregnancy.

PhD Student

O-4

Spatial Gene Expression of Human Coronary Arteries Revealed the Molecular Features of Diffuse Intimal Thickening in Explanted Hearts

Li, Boaz*; Leung, Samuel*; Elishaev, Maria; Cheng, Wan Hei; Mocci, Giuseppe; Björkegren, Johan L. M.; Lai, Chi; Singh, Amrit; Wang, Ying

University of British Columbia

Background: Diffuse intimal thickening (DIT) is a pre-clinical stage of atherosclerosis characterized by thickened intima. The molecular basis of its susceptibility to atherogenesis is unknown, and mechanistic investigations cannot be performed in commonly used mouse models, in which DIT does not exist. Vascular smooth muscle cells (SMCs) are the predominant cell type that occupies the intima and media of DIT. The molecular differences between these two layers may reveal the earliest phenotypic changes in SMCs to promote atherosclerosis. Spatial transcriptomics would allow for exploration of the intima and medial layers separately, which is especially useful for comparing molecular features between intimal and medial SMCs in DIT. As input, there are two types of samples that can be used: autopsy or explanted. Human coronary arteries from autopsies are easily accessible compared to those collected from explanted hearts, however RNA degradation in autopsy samples may introduce bias in the analysis and further complicate data interpretation. Before more studies pursue spatial transcriptomics to understand the etiology of coronary artery disease, the potential pitfall of using autopsy samples needs to be investigated.
Hypothesis: RNA degradation from autopsy samples make it inadequate for downstream spatial transcriptomic analysis.
Methods and Results: Human coronary lesions were obtained from autopsies (n=7) and explanted hearts from transplant patients (n=7), preserved as FFPE blocks, and classified as diffuse intimal thickening (DIT) or pathologic intimal thickening (PIT) using the modified American Heart Association criteria. RNA was extracted (Qiagen, CAT#73504) and quality assessed by DV200 (RNA 6000 Pico Kit, Agilent, CAT#5067-1513) on a Bioanalyzer. DIT samples with DV200 ≥ 30% were sectioned, mounted on Visium SD slides (10× Genomics), stained with H&E, and processed using Seurat (v5.0.1). RNA quality was further examined via spatial heatmaps of mitochondrial gene distribution. Data were normalized with regularized negative binomial regression and batch-corrected using Harmony. Capture spots for intima and medial layers were identified by overlaying them onto H&E images. A linear mixed-effects model identified DEGs between autopsy and explanted samples (intima + medial layers), and within explanted samples between layers. Significance was set at FDR < 0.01. DEGs were ranked by log2-fold change, visualized by volcano plots, and analyzed for gene ontology (Cellular Component 2023) and pathway enrichment (BioPlanet 2019) using Enrichr. Although autopsy samples met the RNA quality standard for Visium (DV200 ≥ 30%), only arteries from explanted hearts exhibited reliable sequencing performance. Genes enriched in TGF-β-mediated remodeling of the extracellular matrix were overrepresented in the intima. SMCs enriched in the intima are dedifferentiated, but unlike those in the atherosclerotic lesions, they are not pro-inflammatory.
Conclusions: Our findings indicate that autopsy samples are not ideal to distinguish subtle differences among cell phenotypes. SMCs in thickened intima may lead to lipid retention but not necessarily the onset of atherosclerosis.

Masters Student

O-5

Lipoprotein(a) and the Arterial Endothelium: Elucidating Mechanisms of Residual Cardiovascular Risk

Andria Henry [1,2], Ruoqi Wang[3], Yitong Liu[3], Ho Man Ryan Yee [4], Michael Boffa[5], Marlys Koschinsky[6], Changsen Wang[2], Warren L. Lee[1,2,4,7]

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[5] Department of Biochemistry and Robarts Research Institute, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada
[6] Robarts Research Institute, University of Western Ontario, London, Ontario, Canada
[7] Department of Medicine and the Interdepartmental Division of Critical Care Medicine, University of Toronto, Ontario, Canada.

Background: Atherosclerosis is initiated by the deposition of lipids, particularly low-density lipoprotein (LDL), underneath the arterial endothelium. Despite effective treatments to significantly lower LDL levels, patients continue to experience cardiovascular events indicating the existence of residual risk factors. Lipoprotein(a) [Lp(a)] is a unique lipoprotein that is structurally similar to LDL but significantly more atherogenic. Both LDL and Lp(a) include the surface apolipoprotein B100, however, Lp(a) is uniquely characterized by the addition of apolipoprotein(a) [apo(a)], which exists in differently sized isoforms. Lp(a) can also bind to oxidized phospholipids, inducing inflammation, which then exacerbates its pro-atherogenic effects. Previous studies have shown that Lp(a) can accumulate in human arteries, with the degree of deposition correlating with the severity of atherosclerosis. However, the mechanism(s) by which circulating Lp(a) in arterial blood crosses the endothelial barrier to deposit in the arterial intima remain unknown.
Hypothesis: We hypothesized that Lp(a) crosses the arterial endothelium by transcytosis using specific receptors.
Methods and Results: Using Total Internal Reflection Fluorescence (TIRF) microscopy, we observed that fluorophore-tagged Lp(a) undergoes transcytosis across primary human coronary artery endothelial cells (HCAEC). Interestingly, Lp(a) undergoes endothelial transcytosis at a higher rate in female HCAECs than male HCAECs. Excess unlabelled Lp(a) and unlabelled LDL both attenuated Lp(a) transcytosis, suggesting potentially common receptors. Knockdown of ALK1 and caveolin-1 significantly attenuated Lp(a) transcytosis, while depletion of LDLR or SR-BI did not. The requirement for ALK1 was observed using Lp(a) from two different donors exhibiting different lengths of apo(a). In addition, an antibody to the ALK1 extracellular domain significantly inhibited Lp(a) internationalization and transcytosis by HCAEC. Finally, Lp(a), but not LDL, activated SMAD1/5 signaling (known to be downstream of ALK1) in HCAEC. In mice, we observed acute and dose-dependent deposition of fluorophore-tagged Lp(a) in the atheroprone region of murine aortas, without deposition of equimolar amounts of dextran (control for paracellular leakage).
Conclusions: Lp(a) can undergo receptor-mediated endothelial transcytosis via caveolae and the ALK1 receptor. Our preliminary findings suggest that Lp(a) may directly bind and activate ALK1 signaling. We are currently determining the molecular mechanisms behind both phenomena in vitro and validating our results in wild type vs tissue-specific knockout animals. Ultimately, this work will elucidate the mechanisms behind Lp(a)-driven atherogenesis and residual cardiovascular risk.

PhD Student

O-6

ApoA1 and HDL Selectively Protect the Heart During Doxorubicin Chemotherapy via Hepatic SR-B1

Jeong-Ah Yoo, Bernardo Trigatti

Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada

Background: Doxorubicin (DOX) is a powerful chemotherapy drug but can cause cardiotoxicity, a life-threatening side effect. Our lab found that either over-expression or injection of Apolipoprotein (Apo) A1, a major component of high density lipoprotein (HDL), protects mice against DOX-induced cardiotoxicity, and that this protection was dependent on the scavenger receptor class B type I (SR-B1) an HDL receptor expressed in a variety of tissues including the liver and cardiomyocytes. Given the similar mechanisms by which DOX induces toxicity in tumor cells and cardiomyocytes, it is critical to test the selectivity of any potential cardioprotective agent for DOX-induced cardiotoxicity. We therefore aimed to determine whether ApoA1 or HDL mediated protection against DOX-induced cytotoxicity was selective for the heart as compared to breast tumor cells, and to explore the requirement for SR-B1 expression in the liver versus cardiomyocytes.
Hypothesis: We hypothesized that ApoA1 and HDL protect against DOX-induced cardiotoxicity in a manner that is selective for the heart and does not compromise the anti-tumor activity of DOX. Furthermore, we hypothesized that this selective cardioprotection requires SR-B1 expression in hepatocytes, but not in cardiomyocytes.
Methods: In vitro, neonatal mouse cardiomyocytes and murine 4T1 breast cancer cells were treated with DOX±HDL or ApoA1 and apoptosis was measured using TUNEL staining. In vivo, Balb/c mice were inoculated with 4T1 tumor cells and treated weekly with DOX alone or in combination with HDL or ApoA1 for 5 weeks. Tumor size over the course of the 5 weeks, and cardiac cell apoptosis and cardiomyocyte atrophy were measured 1 week after the end of the treatment period. To investigate the requirement for SR-B1 expression in hepatocytes versus cardiomyocytes, Liver specific SR-B1 KO mice (SR-B1-LIVKO), cardiomyocyte specific SR-B1 Ko mice (SR-B1-CMKO) or control (SR-B1-floxed) mice (all on C57BL6/J background) were treated weekly for 5 weeks with DOX alone or in combination with ApoA1 and cardiac cell apoptosis and cardiomyocyte atrophy were measured 1 week following the final treatment.
Results: In vitro treatment with HDL but not ApoA1 protected cardiomyocytes against DOC induced apoptosis and atrophy but did not protect 4T1 tumor cells against DOX-induced apoptosis. In vivo, treatment of tumor bearing Balb/C mice with either ApoA1 or HDL protected them against DOX-induced cardiac cell apoptosis and cardiomyocyte atrophy but did not interfere with DOX-induced suppression of tumor growth. Liver specific inactivation of SR-B1 gene expression increased susceptibility of cardiac cells to DOX-induced apoptosis and reduced the effectiveness of ApoA1 injection to suppress DOX-induced cardiac cell apoptosis and prevented ApoA1-mediated protection against DOX-induced cardiomyocyte atrophy. In contrast, cardiomyocyte-specific inactivation of SR-B1 gene expression did not impact ApoA1-mediated protection against DOX-induced cardiac cell apoptosis or cardiomyocyte atrophy.
Conclusions: HDL-based therapies may reduce DOX-induced cardiotoxicity without impacting the effectiveness of DOX-mediated chemotherapy, and this selective cardioprotective effect appears to be dependent on hepatic expression of the HDL receptor, SR-B1.

PhD Student

O-7

Hepatocyte Prosaposin Deficiency Alters Fibrotic Remodeling in a Sex-Specific Manner during Metabolic Dysfunction-Associated Steatohepatitis (MASH) Progression

Aaron Getachew, Jiabei Zheng, Peter U. Amadi, Govind S. Gill, Suha Jarad, Raj Patel, Rong Li, Hongmei Gu, Dawei Zhang

University of Alberta

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a leading cause of chronic liver disease, encompassing a spectrum of liver disorders ranging from simple fat accumulation to its progressive and inflammatory stage, metabolic dysfunction-associated steatohepatitis (MASH). A key determinant of morbidity and mortality in MASH is the development of liver fibrosis, which strongly predicts progression to advanced stages of cirrhosis and hepatocellular carcinoma. Liver fibrosis arises when chronic injury and inflammation disrupt normal repair processes, leading to excessive extracellular matrix (ECM) deposition. Hepatocytes play a central role in this process. During MASH, hepatocytes undergo injury and death, releasing inflammatory mediators and signals that recruit immune cells and activate hepatic stellate cells, driving excess ECM deposition and fibrosis. Recent studies suggest that the lysosome and sphingolipid metabolism are essential for maintaining hepatocyte homeostasis under metabolic stress. Prosaposin (PSAP) is a lysosomal precursor protein required for glycosphingolipid degradation. PSAP has been implicated in stress pathways and cell survival. However, its role in hepatocyte-driven fibrogenesis during MASH has not been explored.
Hypothesis: We hypothesize that prosaposin (PSAP) plays a role in maintaining hepatocyte homeostasis during MASH, and its loss will promote altered extracellular matrix remodelling and exacerbate liver injury.
Methods and Results: The study included two groups of mice: control mice with the floxed Psap gene (Psap fl/fl) and hepatocyte-specific prosaposin knockout mice (PsapHepKO). Male and female mice from both groups were fed a MASH diet for 30 weeks to induce MASH. Fasting body weight, fasting blood glucose, and plasma ALT activity were measured. Fibrosis was evaluated by picrosirius red staining for collagen deposition, and ECM proteins and gene expression (Col1a1, Fn1, Acta2) were analyzed. Inflammatory cytokines Il1b and Tnfa were also assessed. Our findings revealed that endpoint fasting body weight was similar in both sexes; however, females exhibited significantly higher fasting blood glucose (5.9 mmol/L vs 5.1 mmol/L, $p<0.05$) and increased liver weight relative to controls ($p<0.05$), whereas no changes were observed in males. Plasma ALT activity was significantly increased in PsapHepKO mice (5.95 U/L) versus controls (2.00 U/L), representing approximately a 3-fold elevation in both sexes. From the histological analysis, female PsapHepKO demonstrated a significant increase in collagen deposition ($p<0.05$) while males displayed an upward trend. Compared to the control, the expression of ECM gene markers Col1a1 and Acta2 remained unchanged for both sexes. However, both insoluble and plasma fibronectin (Fn1) were significantly decreased ($p<0.05$) in female PsapHepKO. Inflammatory cytokines Il1b and Tnfa gene expression was unchanged between groups.
Conclusions: PSAP deficiency in hepatocytes led to female-specific collagen accumulation, accompanied by reduced Fn1 expression and decreased plasma fibronectin. Additionally, hepatocyte-specific PSAP removal altered metabolic and injury parameters in a sex-specific manner. These findings suggest that hepatocyte-derived PSAP plays a sex-specific role in regulating fibrotic pathways during MASH progression.

Masters Student

O-8

Deciphering the role of retinoic acid signaling in alcohol-associated liver disease using AlbCre:RARdn mice

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Background: Chronic alcohol consumption is a global health burden, predominantly damaging the liver, leading to alcohol-associated liver disease (ALD). Despite significant studies, a comprehensive understanding of the ALD pathogenesis is unclear, and there is no FDA-approved drug available for its treatment. Historically, ALD is associated with disrupting hepatic vitamin A homeostasis, which exerts physiological effects via retinoic acid (RA) signalling in the body.
Hypothesis: We hypothesize that alcohol misuse dysregulates hepatic RA signaling, thereby promoting the pathogenesis of ALD. To test this hypothesis, we propose that transgenic (AlbCre:RARdn) mice, which lack functional hepatic RA signaling, will exhibit exacerbated ALD when fed an alcohol-containing diet.
Methods: AlbCre:RARdn transgenic and control mice were provided with liquid diets with or without alcohol using the NIAAA chronic-binge model of ALD. Hepatic steatosis and injury were evaluated using histopathological and biochemical approaches. Hepatic and plasma Vitamin A levels were measured using HPLC, while hepatic inflammation and lipid metabolism markers were assessed by qPCR and western blot analysis.
Results: AlbCre:RARdn mice exhibited increased hepatic triglyceride content and elevated plasma ALT levels compared to control mice, independent of alcohol consumption. Hepatic cholesteryl ester levels were also higher in transgenic mice compared to control mice. Free fatty acids were increased in alcohol feeding, regardless of genotype. Among the phospholipid species, alcohol increased the levels of phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol, whereas phosphatidylcholine was unaffected by either alcohol or genotype. Although main genotype effects were detected for phosphatidylethanolamine and phosphatidylserine levels, in the post-test, there was no pairwise difference. Hepatic retinol level was unchanged, while retinyl ester levels were decreased, and plasma retinol level was increased in transgenic mice compared to control mice. Hepatic Ppara expression was reduced in transgenic mice, while Pparg expression was unaffected by either alcohol or genotype. Alcohol feeding decreased Srebf1 expression. Tnfa and Col1a1 expression were elevated in transgenic mice compared to controls, but the post-test comparison for Col1a1 showed no pairwise difference between the groups for alcohol exposure. In contrast, Tgfb1 expression was induced by alcohol exposure.
Conclusions: AlbCre:RARdn mice exhibit a pronounced genotype effect characterized by increased hepatic lipid accumulation and elevated liver injury markers. Contrary to our expectations, adding alcohol to the diet did not worsen the ALD in these mice. Future studies on investigating the role of RA signaling in VLDL assembly and export in the context of ALD are warranted.

PhD Student

O-9

Arylacetamide Deacetylase (AADAC) Regulates Hepatic Lipid Hydrolysis and De Novo Lipid Synthesis

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Background: Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) is characterized and initiated by the accumulation of triacylglycerols (TG) and cholesteryl esters (CE) in the liver. Although it is well established that dysfunction of lipase(s) in hepatic lipid turnover is related to the development of MASLD, the regulation of hepatic lipolysis is not fully understood. Furthermore, enzymes catalyzing hepatic CE hydrolysis at neutral pH in hepatocytes are still unknown. Arylacetamide deacetylase (AADAC) is an ER-localized type II membrane protein and was also identified in liver lipid droplet proteome. Our studies have shown that AADAC catalyzes TG and CE hydrolysis, and AADAC deficient mice present with increased TG and CE concentrations in the liver and increased de novo lipogenesis in hepatocytes. Understanding the molecular mechanisms underlying AADAC function is crucial for elucidating the role of AADAC in hepatic lipid metabolism.
Hypothesis: The transmembrane domain of AADAC regulates TG and CE access to the active site of the lipase, and the product released by AADAC in the ER regulates de novo lipid synthesis.
Methods: Protein structure prediction by AlphaFold revealed tyrosine residues in the transmembrane domain of AADAC that interact with the lid helix which may regulate substrate access to the active site. As part of a putative inverted Cholesterol Recognition/interaction Amino acid Consensus (CRAC) motif, these tyrosine residues may mediate regulation of AADAC activity by cholesterol. Esterase activity of AADAC was measured after site-directed mutagenesis of these tyrosine residues and after modulation of membrane cholesterol concentrations. To explore the global impact of AADAC ablation on hepatic lipid metabolism, we performed RNA sequencing (RNA-seq) to generate a comprehensive transcriptomic profile of livers collected from WT and AADAC KO mice after one week of high-fat, high-cholesterol western-type diet feeding.
Results: Mutagenesis of tyrosine residues eliminated esterase activity of AADAC, showing their important role in the catalytic function. Cholesterol depletion in AADAC expressing cells increased its esterase activity, while increased cholesterol concentration reduced the activity, suggesting that ER cholesterol is an important regulator of AADAC activity. Lipogenic genes, as well as pathways that support de novo lipid synthesis, were systematically upregulated in the liver of AADAC KO mice.
Conclusions: We demonstrated that AADAC is a lipase responsible for performed neutral lipid turnover in the liver. Additionally, AADAC may regulate de novo lipogenesis by modulating ER cholesterol concentration through its CE hydrolase activity.

Other Research Staff

O-10

Targeting IP3R1, the major ER calcium release channel in the liver, as a means of lowering PCSK9 expression and secretion

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Background: Hyperlipidemia is a well know risk factor for cardiovascular disease (CVD), the leading cause of death worldwide. Sedentary lifestyles, genetic variations and unhealthy diets, can increase circulating levels of low-density lipoprotein (LDL) cholesterol, thereby promoting CVD. An effective therapeutic strategy involves a lowering in the expression and secretion of proprotein convertase subtilisin/kexin type 9 (PCSK9) from liver cells, thereby improving liver-mediated LDL cholesterol clearance from the blood. We have now developed a novel class of caffeine-inspired therapeutics that potently block PCSK9 expression/secretion. Although caffeine and our lead compounds increase hepatic endoplasmic reticulum (ER)-calcium levels, which initiates the cascade of events that inhibit PCSK9, the caffeine-mediated target and underlying molecular mechanism remains unclear. In this study, we demonstrate that modulating the ER calcium efflux channel inositol 1,4,5-triphosphate receptor (IP3R1), a known target of caffeine, can impact PCSK9 expression/secretion.
Hypothesis: We hypothesized that liver-specific deletion of IP3R1 would mitigate ER-calcium efflux and result in attenuated PCSK9 expression/secretion.
Methods: The immortalized human hepatocyte cell line, HuH7, known to express and secrete PCSK9, was treated with siRNA-mediated knockdown of IP3R1 both in the absence or presence of caffeine to assess their ability to secrete PCSK9 using an established ELISA. CRISPR/Cas9 was then employed to generate IP3R1 knockout (KO) HuH7 cells to confirm whether IP3R1 is the caffeine-mediated target for PCSK9 expression/secretion. Male wild-type (WT) and IP3R1 liver-specific knockout (LSKO) mice (C57BL/6J background) were also studied at 12-weeks of age (~25 g body mass). Both groups received intraperitoneal (IP) injections of vehicle or caffeine (50 mg/kg, 12 hours) to further elucidate the role of IP3R1 in murine hepatic tissue.
Results: We found that siRNA knockdown of IP3R1 led to a 50% reduction in IP3R1 protein levels in HuH7 cells which correlated with a 50% reduction in secreted PCSK9. Caffeine further reduced the secretion of PCSK9 in siIP3R1-treated cells in a dose-dependent fashion. Notably, CRISPR/Cas9-mediated ablation of IP3R1 protein in HuH7 cells decreased PCSK9 secretion by more than 90%, with the addition of caffeine having no effect on further lowering PCSK9. A >80% reduction in PCSK9 mRNA was also observed in the IP3R1 KO cells. Consistent with these findings, serum PCSK9 levels were >75% lower in IP3R1 LSKO versus WT mice. Relative to the WT vehicle group, secreted PCSK9 was significantly reduced by ~50% in WT animals following IP injection of caffeine, whereas PCSK9 levels did not change between conditions in LSKO mice.
Conclusions: We have synthesized novel caffeine derivatives that markedly reduce PCSK9 expression/secretion in cultured hepatocytes and in mice. Collectively, our results suggest that targeting IP3R1 in hepatocytes with our lead derivatives may represent a novel therapeutic strategy for lowering PCSK9 and the management of hyperlipidemia.

Postdoctoral Fellow

O-11
Disrupted AMPK-mTORC1 Signaling Alters Liver Metabolism in Mice

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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is one of the most prevalent chronic liver diseases characterized by liver steatosis, steatohepatitis and fibrosis, and it is linked to an increased risk of cardiovascular disease. MASLD is a growing contributor to end-stage liver disease and liver cancer. Dysregulation of glucose and lipid homeostasis is a key driver of MASLD progression. The AMP-activated protein kinase (AMPK) and mechanistic target of rapamycin complex 1 (mTORC1) are well-established regulators of metabolic processes, which can become dysregulated during MASLD progression.
Hypothesis: While it has been known for almost 20 years that AMPK can inhibit mTORC1 signaling via phosphorylation of the tuberous sclerosis complex 2 (TSC2) and the regulatory-associated protein of mTOR (RAPTOR), the physiological importance of this signaling axis has never been explored. We hypothesized that disrupting endogenous AMPK signaling to mTORC1 will lead to increased chronic mTORC1 activation that will increase liver lipogenic programs.
Methods: We generated TSC2 S1345A/RAPTOR S722/792A double knock-in (DKI) mice using CRISPR-Cas9. To begin to understand the importance of this axis, we performed fasting and refeeding experiments where the lipogenic and gluconeogenic responses are well characterized. Blood and tissue samples were collected to assess metabolic changes at physiological, transcript and protein levels.
Results: Levels of serum insulin, glucose and ketone bodies in fasting and refed mice showed no difference between genotypes. Liver transcript analysis uncovered a sex-specific response in lipogenic transcript expression with no changes in gluconeogenic transcripts. Following the refeeding, female DKI mice exhibited significantly lower expression of lipogenic transcripts compared to WT controls, whereas these differences were absent in males. Immunoblotting of liver lysates revealed different phosphorylation of Ribosomal Protein S6 between WT and DKI mice in both sexes, which is an important marker of mTORC1 activity. In line with the transcript profile, the expression of lipogenic relevant proteins suggested an altered lipogenesis in refed DKI mice, which was also sex dependent. Gluconeogenic protein levels remained unchanged between WT and DKI mice.
Conclusions: Our findings suggested that while hepatic gluconeogenesis remains unaffected, lipogenesis may be significantly modulated by alterations in AMPK-mTORC1 signalling, highlighting a potential effect of chronic mTORC1 activation on lipid metabolism. This work may help provide insights into the relationship between the AMPK-mTORC1 crosstalk and MASLD, and may be useful in determining mechanisms of metabolic therapies such as intermittent fasting and metformin.

Masters Student

O-12

Understanding the role of Irx1 and Irx2 transcription factors in pancreatic alpha cell identity

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Background: Diabetes is a chronic metabolic disorder that imposes a significant health and economic burden in Canada and worldwide. Its central pathological feature is pancreatic islet dysfunction. While most research has focused extensively on pancreatic beta cells and insulin secretion, pancreatic alpha cells are also dysregulated in both type 1 and type 2 diabetes. Diabetic alpha cells exhibit hyperproliferation, transcriptional remodeling, and impaired glucagon secretion. Despite their noted contributions to metabolic disease, our current understanding of the regulation and function alpha cells in metabolic dysregulation remains comparatively underexplored. Iroquois homeobox transcription factors, Irx1 and Irx2 (Irx1/2), are specifically expressed in pancreatic alpha cells during development and adulthood and conserved in mice and humans. However, their roles in alpha cell biology have not previously been investigated.
Hypothesis: We hypothesized that Irx1/2 are required for the maintenance of alpha cell identity and function.
Methods and Results: Single-cell transcriptomic analysis of pancreatic alpha cells from diabetic mice and human donors revealed significant downregulation of Irx1/2 expression, suggesting potential implications of Irx1/2 in altered transcriptional programs and cellular responses of the pancreas in diabetes. To investigate their functional role in the adult pancreatic alpha cells, we utilized alpha cell-specific Irx1/2 double knockout mice (Irx1/2ADKO) generated by crossing Irx1flox/flox:Irx2-/- line with Gcg-CreERT2 mice. To bypass the potential developmental effects, Cre-Lox recombination was induced by tamoxifen injections at 8 weeks of age in male and female mice. Irx1/2ADKO mice and littermate control mice on a standard chow exhibited comparable body weight and body composition. In addition, glucose tolerance, insulin tolerance, and pyruvate tolerance tests performed four weeks after recombination revealed no significant differences between Irx1/2ADKO mice and wild-type controls, indicating preserved systemic metabolic function without metabolic stress or intervention. However, plasma glucagon levels measured in fed and fasted states were significantly reduced in Irx1/2ADKO mice compared to controls. While no overt morphological abnormalities were noted in H&E-stained pancreatic sections, immunofluorescent staining revealed markedly reduced glucagon expression without changes in insulin expression in Irx1/2ADKO pancreas compared to controls, corroborating the functional phenotypes (i.e. decreased plasma glucagon levels). To further interrogate alpha cell identity by Irx1/2 depletion, I performed Gcg-CreERT2-based lineage tracing analysis by crossing Irx1/2ADKO mice with Rosa26tdTomato. Notably, the loss of Irx1/2 led to an increased number of tdTomato+ cells, and these Gcg-lineage cells variably lacked glucagon but expressed insulin, suggesting potential conversion of adult pancreatic alpha cells to beta cells upon Irx1/2 loss.
Conclusions: Collectively, our findings suggest Irx1/2 as novel regulators of mature alpha cell fate. Particularly, the potential conversion of alpha cells to beta cells by loss of Irx1/2 highlights their importance in diabetes pathophysiology. These results provide mechanistic insight into islet cell plasticity. Ongoing experiments, including perifusion assays to assess islet secretory function and single-cell RNA sequencing to define transcriptome-based cell identity and trajectories, will further elucidate the cellular and molecular mechanisms of pancreatic Irx1/2.

Masters Student

O-13

The Vagus Nerve Mediates NTS Glucagon Signalling to Lower Triglyceride-Rich Very Low-Density Lipoprotein Secretion

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Background: Glucagon regulates lipid metabolism through direct actions on peripheral organs such as the liver and adipose tissue, as well as in the brain. In particular, the nucleus of the solitary tract (NTS) in the brainstem is known to regulate lipid and energy metabolism. In non-diabetic rats, NTS glucagon, requiring NTS glucagon receptor and PKA, reduces hepatic triglyceride-rich very low-density lipoprotein (VLDL-TG) secretion. In a rat model of type 2 diabetes (T2D), direct PKA activation within the NTS, but not glucagon itself, reduces VLDL-TG secretion. The hepatic vagus nerve is a key conduit for brain-liver communication to regulate hepatic lipid and glucose metabolism, but its role in mediating the hypolipidemic effects of NTS glucagon remains unexplored.
Hypothesis: We hypothesized that the hypolipidemic effects of NTS glucagon and NTS PKA activation on hepatic VLDL-TG secretion in non-diabetic and T2D animals, respectively, are mediated via the hepatic vagus nerve.
Methods and Results: Male Sprague-Dawley rats underwent stereotaxic NTS bilateral cannulation, vascular catheterizations, and hepatic vagotomy or sham vagotomy (control) surgery to investigate the role of the hepatic vagus nerve. T2D was induced using nicotinamide and streptozotocin injections combined with high-fat diet-feeding, confirmed by daily blood glucose monitoring. Hepatic VLDL-TG secretion was assessed in 10h-fasted rats following intravenous injection of poloxamer (a lipoprotein lipase inhibitor) with concurrent NTS infusions of glucagon, or PKA activator (Sp-cAMPS), or vehicle control. In non-diabetic rats, NTS glucagon lowered VLDL-TG secretion in sham-operated rats. Vagotomy abolished the hypolipidemic effect of NTS glucagon, demonstrating the requirement of vagal neurotransmission to mediate the regulation of VLDL-TG by NTS glucagon. In T2D rats, NTS Sp-cAMPS infusion lowered VLDL-TG secretion in sham T2D rats, but this effect was abolished in vagotomized rats. These changes occurred independently of plasma glucose, insulin, glucagon, and apoB48/100 levels, but the reductions in VLDL-TG in sham-operated rats given NTS glucagon or NTS Sp-cAMPS correlated with reduced plasma free fatty acids.
Conclusions: Our findings suggest that the VLDL-TG-lowering effect of NTS glucagon in non-diabetic and T2D animals requires an intact hepatic vagus nerve, as NTS glucagon and PKA activation in the NTS reduces VLDL-TG secretion in a vagus-dependent manner. These results highlight the hepatic vagus nerve as a critical mediator of NTS glucagon's lipid-lowering effects.

Masters Student

O-14

The association of healthy eating index score and n-3 fatty acid intake with cardiovascular diseases incidence and lipid biomarkers in Alberta's Tomorrow Project cohort

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Introduction: Unhealthy diet and dyslipidemia are major risk factors for cardiovascular disease (CVD). Studies have shown an inverse association between greater n-3 fatty acid (FA) intake and reduced dyslipidemia and CVD risk. Recent data from Alberta's Tomorrow Project (ATP) demonstrated that individuals with incident CVD (versus without) had higher non-fasting remnant cholesterol (RC). We aimed to assess the association of the healthy eating index (HEI) score and n-3 FA intake with CVD incidence and non-fasting RC in the ATP cohort.
Methods: This is a prospective study on ATP study participants. The study was conducted in a subset of ATP participants with dietary data (n=23,248), 36% male and 64% female, mean age of 50.2 (35-69) years, and no history of cancer or CVD in Alberta, Canada. Dietary intake was assessed using the Canadian Diet History Questionnaire (CDHQ), a food frequency questionnaire (FFQ) from which the Canadian HEI-2005 score and total n-3 FA intake were calculated. Lipid panel markers were measured from non-fasting blood samples, and CVD was defined using the International Statistical Classification of Diseases and Related Health Problems from linked administrative health records. The two-sample independent t-test was used to compare the means between groups. The Cox proportional hazard model was used to assess the association of dietary intakes with CVD incidence, and the linear regression was used to assess the association of dietary intakes with lipid biomarkers.
Results: The mean follow-up was 13.9 years. CVD incidence in males was significantly higher than females (28 vs 22 %, $P<0.001$). For every 1 unit increase in the HEI score, the adjusted Hazard Ratio (HR) of developing CVD decreased [HR:0.98 (95% confidence interval (CI) 0.97-0.98), 0.99 (95%CI 0.98-0.99), and 0.97 (95%CI 0.97-0.98) in females, males, and total cohort, respectively) ($P<0.05$). No significant association was found between absolute n-3 FA intake (g/d) with CVD incidence in males [HR=1.09 (95%CI 0.99-1.2)], females [HR=1.09 (95%CI 0.91-1.11)], or the total group [HR=1.05 (95%CI 0.98-1.12)]. However, higher relative intake (i.e. n-3 FA as proportion of energy) increased the risk of developing CVD [HR=1.42 (95%CI 1.1-1.84), P=0.006] in males. Adjusted multivariate regression in a subset (n=8,458) showed no association between n-3 FA (g/d) intake and lipid biomarkers but a significant inverse association between HEI score and non-fasting RC [coefficient: -0.006 (95%CI -0.009- -0.003) for females and -0.01 (95%CI -0.018- -0.005) for males], and TG levels [-0.01 (95%CI -0.015- -0.006) for females and -0.01 (95%CI -0.02- -0.006) for males].
Conclusions: Higher overall diet quality but not n-3 FA intake was associated with a lower risk of CVD incidence and non-fasting RC. Maintaining a high-quality diet is important in the prevention of dyslipidemia and CVD.

PhD Student

O-15

Lysophosphatidylcholines as early markers and molecular mediators of AMI-induced cardiogenic shock

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Background: Cardiogenic shock (CS) is a severe manifestation of acute heart failure leading to circulatory collapse, with in-hospital mortality approaching 50% despite advances in therapeutic options. This persistently high mortality is driven by the lack of specific biomarkers for early detection and a poor understanding of the systemic collapse that follows a drop in cardiac output. These shortcomings often lead to delays in treatment, exacerbating patient morbidity and mortality. Given the rapid metabolic shifts during CS, we investigated plasma lipidomic alterations in acute myocardial infarction (AMI)-induced CS in a hemodynamically well-defined patient cohort. We further used a large animal model to define the temporal and spatial patterns of these lipid changes and determine whether they originate from cardiac tissue or peripheral sources.
Hypothesis: There is a distinct lipidomic signature that will define AMI-induced CS.
Methods: Plasma and comprehensive hemodynamic data were collected from 259 patients presenting with ST-elevation myocardial infarction (STEMI) prior to intervention. A porcine model of AMI-induced CS was used for temporal and spatial lipid analysis, with serial blood sampling from systemic venous, coronary sinus, and arterial sites. Lipidomic profiling was conducted using liquid chromatography coupled with tandem mass spectrometry. Enzyme-linked immunosorbent assays were used to assess concentrations of enzymes related to important lipid class changes.
Results: Of 310 analyzed lipids, several lysophosphatidylcholine (LPC) species were inversely correlated with lactate, while acylcarnitines were positively correlated with lactate. Heart rate, stroke index, the Granov Goor Index (GGI, a surrogate for left ventricular function), and left ventricular stroke work index demonstrated significant correlations with LPC levels, whereas the classically used parameters of blood pressure, cardiac output, and cardiac index showed limited or no correlation with the plasma lipid profile. Furthermore, lower LPC levels at presentation were associated with increased adverse outcomes and in-hospital mortality, and LPC levels were independent predictors of patient mortality. Levels of secretory phospholipase A2-IIa (sPLA2-IIa), a key enzyme responsible for LPC synthesis, were paradoxically inversely related to LPC levels. Autotaxin, an enzyme responsible for LPC cleavage, was also negatively associated with LPC levels. In the porcine model, LPCs declined progressively during AMI-induced CS, most notably in peripheral venous blood. Flux analysis indicated that baseline LPC release shifts toward systemic consumption or catabolism during ischemia-related shock.
Conclusions: Traditional CS parameters correlated poorly with lipidomic changes, whereas reduced LPC levels closely tracked worsening hemodynamics, elevated lactate, and mortality risk. The inverse associations of LPC with autotaxin and sPLA2-IIa suggest a regulatory feedback loop in LPC metabolism. Animal model data indicate that LPC changes are largely systemic rather than cardiac in origin. LPCs emerge as promising metabolic and hemodynamic biomarkers for AMI-related CS and potential targets for future therapeutic interventions.

PhD Student

O-16

Extreme HDL and risk for cardiovascular disease and mortality: An ancestry-based analysis

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Background: The prognostic significance of extreme high-density lipoprotein cholesterol (HDL-C) levels remains controversial. While higher HDL-C has traditionally been considered protective against atherosclerotic cardiovascular disease (ASCVD), recent studies suggest a non-linear association, with both extremely low and extremely high HDL-C linked to adverse outcomes. Whether these patterns vary across ancestries, and which specific causes of death account for risk at HDL-C extremes, remains incompletely understood.
Hypothesis: We hypothesized that both extreme low and high HDL are associated with adverse outcomes, modulated by ancestry. In addition, unique causes of death can be identified for each extreme.
Methods: We conducted prospective analyses in two large population-based biobanks: the UK Biobank (UKB; n=419,620) and All of Us (AoU; n=155,485). Participants were free of ASCVD at baseline and classified as Asian, Black, or White using genetic ancestry. Baseline HDL-C concentrations (mmol/L) were modeled using generalized additive models and Cox proportional hazards regression. Categorical cut-points were based on the UKB-specific distribution, defining extremely low (<10th percentile), low (10th–30th), reference (30th–70th), high (70th–90th), very high (90th–97.5th), and extremely high (≥2.3 mmol/L) HDL-C groups. Models adjusted for age, sex, smoking status, lipid- and blood pressure–lowering therapy, type 2 diabetes, hypertension, elevated waist circumference, and deprivation index. The primary outcome was incident ASCVD, defined as a composite of myocardial infarction, stroke, and cardiovascular death. Secondary outcomes included all-cause mortality and, in UKB, cause-specific mortality identified from national death registries. Heterogeneity by ancestry was assessed using interaction terms.
Results: Median HDL-C varied by ancestry and sex, with Asians having the lowest median levels (UKB: 1.21 mmol/L) and Whites the highest (1.40 mmol/L), and females exhibiting higher HDL-C than males in all ancestry groups. HDL-C–ASCVD relationships were significantly non-linear in both cohorts ($p<0.05$). Compared with the reference category, extremely low HDL-C was associated with markedly higher ASCVD risk (UKB HR 2.15, 95% CI 2.06–2.25; AoU HR 2.60), while the top decile had the lowest risk (UKB HR 0.51, 0.47–0.54; AoU HR 0.43). Protective associations at high HDL-C were strongest in Whites, weaker in Blacks, and less precise in Asians. All-cause mortality followed a J-shaped curve, most evident in Whites, with extremely high HDL-C associated with increased mortality (UKB HR 1.31, 1.22–1.40) despite low ASCVD risk. Cause-specific analyses at extremely high HDL-C revealed elevated mortality from chronic obstructive pulmonary disease, lung infections, and ischemic stroke, but not ischemic heart disease. Extremely low HDL-C was linked to higher mortality from ischemic heart disease and cancer.
Conclusions: HDL-C is associated with cardiovascular and mortality outcomes in a non-linear, ancestry-dependent manner. Extremely low HDL-C consistently signals elevated ASCVD and mortality risk, while extremely high HDL-C in Whites is linked to excess, primarily non-ischemic, mortality. Risk assessment tools should avoid assuming monotonic benefit from high HDL-C and should account for ancestry-specific patterns.

Postdoctoral Fellow

O-17

Oxidized phosphatidylcholine induced neurodegeneration in the grey matter is partially dependent on necroptosis activation and is mitigated by microglia

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Background: Multiple sclerosis (MS) is a chronic neuroinflammatory and neurodegenerative disease affecting 2.9 million worldwide. People living with MS (pwMS) experience a range of disabilities including muscle weakness, vision impairment, impaired sensation, and cognitive dysfunction. Accumulating evidence suggest oxidative stress in the central nervous system (CNS) contributes to MS pathology. Notably, oxidative phosphatidylcholines (OxPC), products of lipid peroxidation, are elevated in white matter (WM) and grey matter (GM) lesions found in the brains from pwMS. While we previously found that OxPC deposition in the mouse spinal cord WM induces axonal loss, demyelination, and neuroinflammation, the consequences of OxPC accumulation in the GM during MS remains relatively unknown.
Hypothesis: OxPC deposition in CNS GM induces neuroinflammation and neuronal injury in which microglia play a key modulatory role. Targeting pathways underlying neurodegeneration can ameliorate OxPC-induced neurodegeneration in the GM.
Methods and Results: To determine the consequences of OxPC accumulation in the GM, I stereotaxically deposited POVPC, a purified OxPC found to be elevated in MS brain lesions, into the spinal cord GM of mice and assess lesion pathology after 1, 3, 7, and 14 days using quantitative immunofluorescence microscopy. I found that a one-time OxPC deposition in the GM induced significant NeuN+ neuronal cell loss and accumulation of IBA1+/CD11b+ NADPH oxidase+ reactive microglia/macrophages by day 3 and persisted until day 14. Notably, OxPC were not detected in the GM 1 day post injection and only began to accumulate significantly by day 7, suggesting an endogenous production of OxPC in the GM once injury had been initiated. These results indicate the accumulation of MS-relevant OxPC such as POVPC in the CNS GM directly promotes neuroinflammation and neurodegeneration. Using TMEM119CreER:Ai9TdTomato transgenic mice, I next analyzed the ontogeny of inflammatory microglia/macrophages that responded to OxPC deposition and found that while monocyte derived cells are initially recruited to the GM lesion, microglia are the predominant macrophage population in the lesion by day 7 and 14. Importantly, pharmacological depletion of microglia/macrophage using the CSF1-R antagonist PLX3397 significantly reduced NeuN+ cell survival in GM lesions, which indicates that these cells promote neuroprotection during oxidative injury in the GM. Moreover, I found that neuronal cells have significantly increased immunoreactivity to cleaved-caspase-3 and pMLKL/pRIPK3, suggesting that OxPC induced both neuronal apoptosis and necroptosis, respectively. Finally, pharmacological inhibition of RIPK3 significantly increased NeuN+ cell survival.
Conclusions: Collectively, these findings indicate that OxPC accumulation in the GM of the CNS promotes neuroinflammation and neurodegeneration and may contribute to GM injury during MS. While both monocytes and microglia initially respond to the OxPC insult, microglia increasingly replace monocytes in the site of injury over time and helps to mitigate OxPC-induced neurodegeneration. Finally, OxPC neurotoxicity is partially dependent on the activation of pMLKL and pRIPK3, thus pharmacological inhibition of necroptosis may be an effective strategy to reduce GM injury during MS.

Masters Student

O-18

Dipeptidyl Peptidase-4 (DPP4) at the Crossroads of Metabolism, Inflammation, and Atherosclerosis

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Background: Dipeptidyl peptidase-4 (DPP4) is a protease best known for its role in the regulation of glycemia through the cleavage of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Elevated plasma DPP4 concentrations have been consistently contributed and associated with clinical measurements of metabolic dysfunction and atherosclerosis. Both immune cells and hepatocytes have been identified as important sources of increased DPP4 in response to metabolic dysregulation and inflammation.
Hypothesis: DPP4 expression is stimulated in both macrophages and smooth muscle cells by inflammatory stimuli, while elimination of DPP4 will reduce inflammation within the arterial wall and reduce progression of atherosclerosis.
Methods and Results: We used bone marrow-derived macrophages (BMDMs) polarized with LPS+IFNγ (M1-like/pro-inflammatory) or IL-4 (M2-like/pro-resolving). Dpp4 mRNA expression was ~5.5-fold higher in M1-like macrophages. BMDMs were also treated with aggregated LDL cholesterol (aggLDL) to promote foam cell formation and found that M1-like macrophage Dpp4 gene expression is decreased ~1.7 fold in the aggLDL treated BMDMs. Interestingly, when vascular smooth muscle cells are stimulated with M1-like BMDM conditioned media for 24 hours, Dpp4 gene expression increases ~4.5 fold compared to MΦ-BMDM conditioned media. To evaluate DPP4's functional role, Dpp4+/+ and Dpp4-/- mice were injected with PCSK9-AAV and fed a high-fat high-cholesterol (HFHC) diet to induce hypercholesterolemia and atherosclerosis. Dpp4-/- mice exhibited significantly reduced atherosclerotic plaque area at both 10- and 22-weeks of HFHC feeding compared to wildtype controls, despite no differences in circulating lipid profiles or body weight. Immunofluorescence confirmed the presence of DPP4 in atherosclerotic plaques. Nanostring mRNA pathway analysis of aortic tissue revealed downregulation of Toll-Like Receptor (TLR) signaling in Dpp4-/- mice, suggesting an inflammation-centric mechanism. To add, when mesoscale inflammatory protein profiling was performed using the aortic tissue of these mice, Dpp4-/- mice exhibited downregulation of key TLR-mediated inflammatory proteins well-known for their role in atherosclerosis like MCP-1 and KC.
Conclusions: These results demonstrate that DPP4 may be a key promoter of atherosclerosis through regulating inflammation in macrophages and VSMCs, possibly through the pro-inflammatory TLR pathway rather than lipid accumulation and modulation in the plaques. DPP4 emerges as a key mediator linking metabolic dysfunction to vascular inflammation.

Masters Student

O-19

Targeting PRMT5 limits plaque growth by blunting macrophage proliferation and boosting efferocytosis.

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Lady Davis Institute

Background: In atherosclerosis, local macrophage proliferation and inadequate efferocytosis contribute substantially to lesional buildup and instability. Alternatively spliced genes identified in activated macrophages could contribute to these processes. Regulation of spliceosome components and of RNA-binding proteins (RBPs) by PRMT5 is likely to contribute to alternative splicing in this context. Nevertheless, the role of PRMT5 in atherogenesis, and PRMT5-dependent splicing events that underlie the atherogenic processes, remain undefined.
Hypothesis: We hypothesize that the atherosclerotic milieu primes PRMT5-dependent alternative splicing events that promote cell proliferation and alter macrophage function.
Methods & Results: We generated LysM-Cre/Prmt5fl/fl mice that bear a Prmt5 gene deletion in macrophages and granulocytes (Prmt5dM). PRMT5 expression was reduced in bone marrow cells from Prmt5dM mice compared with fl/fl littermates (Prmt5WT) at both the mRNA and protein levels. Prmt5dM ->Ldlr-/- and Prmt5WT -> Ldlr-/- chimeric mice were produced by irradiation / transplantation studies, whereas Apoe-/-Prmt5dM and Apoe-/-Prmt5WT were bred. Male and female mice were fed a high fat diet for 9 weeks. Total cholesterol and plasma lipids were unaffected by genotype. In Prmt5WT -> Ldlr-/- mice, PRMT5 was extensively expressed throughout the atherosclerotic plaque. However, the obligate binding partner of PRMT5, MEP50, strongly co-localized with Mac2+ monocytes/macrophages, suggesting enhanced PRMT5 activity in these cells. In the Prmt5dM -> Ldlr-/- chimeras, PRMT5 no longer overlapped with Mac2 stain, confirming efficient myeloid PRMT5 deletion. Most importantly, atherosclerotic plaque formation was significantly reduced in male and female Ldlr-/- mice reconstituted with Prmt5dM bone marrow compared with WT bone marrow. Similarly, atherosclerotic lesions were significantly smaller in Apoe-/-Prmt5dM than Apoe-/-Prmt5WT mice. Bone-marrow–derived macrophages (BMDMs) were generated from Prmt5dM and Prmt5WT animals. Both PRMT5 and MEP50 expression were significantly upregulated by oxLDL, pointing to activation of the PRMT5 methylosome during early macrophage reprogramming. Functional assays showed reduced proliferation, increased apoptosis, as well as greater efferocytosis and DiI-oxLDL uptake in Prmt5dM compared with Prmt5WT BMDM. RNA-seq revealed that PRMT5 loss suppressed E2F/G2M and DNA-repair programs while enhancing ECM/adhesion and inflammatory signatures in BMDM. We identified 276 significant alternative splicing events that differed between control and oxLDL BMDM. These were aligned with RNA-binding proteins binding up to 250 bp upstream or downstream of the splice sites. In WT BMDM, the top hit RBP was identified as SRSF1, a well-known PRMT5 methylation target. In Prmt5dM BMDM, however, SRSF1 was not implicated in alternative splicing at all.
Conclusions: The splicing landscape in macrophages is not well known. We have set out to identify targets and substrates of PRMT5 to better understand how PRMT5 may modulate atherosclerosis. Our data help us understand the epigenetic landscape of atherosclerosis which may help identify genetic variants linked to disease susceptibility.

PhD Student

O-20

Early hyperactivation of endothelial-specific pathways prevents diabetes-associated chronic vascular damage

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Background: Cardiovascular disease is the leading long-term complication of both type 1 and type 2 diabetes. It was hypothesized that early hyperglycemia may cause permanent damage to the vascular endothelium and ultimately drive chronic cardiovascular complications. Our laboratory has previously observed that the anti-hypertensive angiotensin II receptor blocker (ARB) telmisartan excels at promoting chronic increases in protective endothelial function and its elusive main mediator, nitric oxide (NO), in pleiotropic, blood pressure-independent fashions. Whether individuals with diabetes can benefit from early NO-dependent endothelial function hyperactivation with telmisartan through improvements in cardiovascular outcomes is unknown.
Hypothesis: Early NO-dependent endothelial function hyperactivation with telmisartan can prevent hyperglycemia-induced chronic vascular damage.
Methods: Hyperglycemic Ins2+/Akita (Akita) mice were compared to age/sex-matched Ins2+/+ (control) littermates. Four weeks after diabetes onset (blood glucose ≥ 16.6mmol/L), mice were treated with telmisartan (10mg/kg drinking water) or vehicle for eight weeks. Prevention of aortic stiffening was quantified through pulse wave velocity (PWV) in vivo, whereas changes in endothelial function were quantified by reduced constriction and improved NO-dependent vasorelaxation via ex vivo myography.
Results: The 1.4-fold increase in PWV observed in hyperglycemic Akita mice over control littermates, a sign of aortic stiffening, was normalized with telmisartan. Following termination, hyperglycemic mice showed 16.2% increases in phenylephrine (PE)-induced smooth muscle constriction and 20.2% ($p<0.01$) reductions in acetylcholine (Ach)-induced endothelial NO-dependent relaxation, two markers of vascular damage, which were partially (36%) and fully (100%) rescued by telmisartan, respectively.
Conclusions: Early intervention with telmisartan prevents aortic stiffness and rescues NO-dependent endothelial function, suggesting telmisartan as a potential therapeutic in reducing cardiovascular damage in diabetes.

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PhD Student

O-21
Characterization of Gut-Derived Extracellular Vesicles during Active Lipid Absorption

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Background: The intestine plays critical roles in nutrient homeostasis and systemic health. The small intestine is the major organ that packages dietary lipids into chylomicrons and secretes them into the mesenteric lymph. Extracellular vesicles (EVs) are secreted by a variety of cells, circulate in biofluids, and function as a mechanism for cell-to-cell communication. EV secretion from the intestine into lymph fluid during fasting and active lipid absorption has not been examined.
Hypothesis: The intestine secretes EVs with distinct signatures during fasting and active lipid absorption.
Methods: Male Sprague-Dawley rats (200–350 g) were surgically implanted with cannula in the mesenteric lymph duct and the duodenum and were randomly assigned to two groups to receive a bolus infusion of either Intralipid or normal saline (n=9 per group). Lymph fluid was collected before and after Intralipid or saline administration. Lymph triglycerides were measured to assess lipid secretion. Size and morphology of EVs in lymph fluids were characterized by transmission electron microscopy and nanoparticle tracking analysis. EVs collected 1 hour before (-1h) and 2 hours after (2h) Intralipid/saline administration were further analyzed by flow cytometry with fluorescence conjugated antibodies against CD63, CD81, CD9 and apolipoprotein B (ApoB).
Results: Compared with saline infusion, Intralipid infusion increased lymph triglyceride output, peaking at 2 hours. EVs were detected in lymph fluids collected both before (-1h) and after (2h) Intralipid/saline infusion, with significant elevations following Intralipids, confirmed by increasing CD63+, CD81+ and CD9+ percentage in flow cytometry. Depletion of chylomicrons increased the percentage of CD9+, but not CD63 and CD81+. In addition, different EV subtypes (classified as CD63/CD81+ and CD9+) exhibited distinct patterns in ApoB+ particles, with the median fluorescence intensity of CD63 and CD81 being significantly higher than that of CD9.
Conclusions: The intestine secretes EVs into mesenteric lymph under both fasting and postprandial conditions. Lipid absorption enhances EV secretion. EVs may bind to and co-secrete with chylomicrons. Chylomicron depletion may assist in EV purification in lipid-rich lymph fluid with limited enrichment effects. Finally, gut-derived EVs during active lipid absorption may include multiple subtypes with different affinities to chylomicrons. The biological functions of gut-derived EVs warrant further studies.

Postdoctoral Fellow

O-22

M30, a Thirty Amino Acid Mid-Segment of Nesfatin-1 Exhibits Potential Lipid Lowering Effects in a Human Liver Cell Line

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Nesfatin-1, an 82-amino acid peptide derived from nucleobindin-2 (NUCB2), has garnered attention for its insulinotropic and metabolic functions. Nesfatin-1 encodes a 30 aa mid-section fragment (M30), whose metabolic effects remain underexplored. We hypothesise that M30 regulates lipid metabolism in the liver by modulating lipid synthesis and beta-oxidation gene expressions, and potentially lowering lipid accumulation. Therefore, using a human liver cell line (HepG2 cell), this study investigates the effect of M30 on lipid metabolism genes. HepG2 cells were treated with Mouse M30 (0.1, 1, 10 nM) or control (no M30) for 24 hours in vitro, followed by qPCR analysis of lipid synthesis and beta-oxidation related genes. Mainly at 10 nM, M30 significantly down-regulated lipid synthesis genes, including acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), HMG-CoA reductase (HMGCR), glycerol-3-phosphate acyltransferase (GPAM), and upregulated diacylglycerol O-acyltransferase 2 (DGAT2), compared to the untreated control ($p < 0.05$). Beta-oxidation genes such as carnitine palmitoyltransferase 1a (CPT-1a) was significantly up-regulated in the treated group. The up- and downregulation of DGAT2 and GPAM, respectively, which are pivotal in triglyceride synthesis, imply that M30 could mitigate hepatic steatosis. These findings suggest that M30 suppresses genes critical in hepatic lipid synthesis pathways. Our current research focuses on determining whether the changes in genes found here translate into a reduction in lipid levels in HepG2 cells. If M30 lowers lipid accumulation in liver cells, it has the potential to be pursued as a therapeutic target for lipid disorders, liver disease, including metabolic dysfunction-associated steatohepatitis (MASH).

Postdoctoral Fellow

O-23

Empagliflozin Ameliorates Hepatic Steatosis and Fibrosis via Ketogenesis-Dependent and -Independent Mechanisms

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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent chronic liver disease, characterized by excessive lipid accumulation that can progress to metabolic dysfunction-associated steatohepatitis, cirrhosis and liver cancer. MASLD is closely linked to metabolic disorders, particularly type 2 diabetes (T2D). Sodium-glucose cotransporter 2 inhibitors (SGLT2i), developed for glycemic control in T2D, have demonstrated clinical benefits in MASLD. Although the underlying mechanism remains unclear, clinical evidence indicates that SGLT2i elevate plasma ketone bodies, which may serve as key mediators of their therapeutic effects.
Hypothesis: We hypothesize that empagliflozin (EMPA), an SGLT2i, modulates hepatic ketogenesis, and that its ketogenic effects contribute to MASLD treatment.
Methods: To evaluate the role of ketogenesis in the therapeutic effects of EMPA against fatty liver disease, we first established MASLD by subjecting mice a high-fat diet for 20 weeks. Then, adult-onset liver-specific Hmgcs2 knockout was induced using AAV-TBG-Cre (Hmgcs2-LKO). Mice were treated with EMPA (10mg/kg) in drinking water for 10 weeks. Body weight and composition were monitored every week. After 10 weeks, liver histology, lipid accumulation, inflammatory markers, and ketone body levels were assessed. RNA sequencing was performed to examine the molecular mechanisms of EMPA.
Results: EMPA improved liver health in MASLD mice, lowering hepatic lipid accumulation and fibrosis, while elevating fasting ketone bodies. Body weight, glucose tolerance and insulin sensitivity were unchanged. Transcriptomics/qPCR analyses demonstrated reduced expression of de novo lipogenesis genes (Fasn, Acaca) as well as immune (F4/80) and fibrotic genes (Col1a1, Col1a2, and Timp1). In contrast, EMPA failed to elevate ketone body levels and ameliorate steatosis in Hmgcs2-LKO mice, with inflammatory and lipid-handling genes remaining unchanged. Notably, EMPA still reduced fibrosis in Hmgcs2-LKO liver with suppression of extracellular matrix-related genes (Mmp2, Smoc2). These findings suggest dual mechanisms of EMPA: (1) hepatic ketogenesis-dependent improvement in steatosis and inflammation, and (2) a largely ketogenesis-independent anti-fibrotic effect.
Conclusions: Our findings identify hepatic ketogenesis as a key driver of EMPA's benefits in MASLD, mediating improvements in steatosis and inflammation. EMPA also attenuated hepatic fibrosis via a pathway only partially linked to ketogenesis, suggesting that therapeutic responses in MASLD are not exclusively ketone-dependent and may involve additional mediators. These insights provide the mechanistic basis for the use of SGLT2i in MASLD and may support broader applications across heart failure and chronic kidney disease.

Postdoctoral Fellow

O-24
GLP-1R/GIPR Dual Agonism Reduces Intestinal Lipid Secretion

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Background: Metabolic diseases (e.g., obesity and type 2 diabetes) are associated with atherogenic dyslipidemia that leads to atherosclerotic cardiovascular disease (ASCVD) and increased mortality. The gut contributes to atherogenic dyslipidemia in these conditions by overproducing lipoprotein particles (chylomicrons). Tirzepatide, a dual agonist of the glucagon-like peptide-1 receptor (GLP-1R) and glucose-dependent insulinotropic polypeptide receptor (GIPR), is an effective treatment for obesity and type 2 diabetes and has also been shown to improve blood lipid profiles and reduce ASCVD risk. However, it remains unclear whether these effects are driven by systemic metabolic changes or direct modulation of lipid metabolism in the gut.
Hypothesis: We hypothesize that GLP-1R/GIPR dual agonism with tirzepatide improves gut lipid handling through modulation of lipid metabolism in the gut. To test this hypothesis, we investigated the effects of short-term tirzepatide treatment on lipid secretion in the gut and examined the underlying molecular mechanisms in a rat model.
Methods and Results: Sprague-Dawley rats were fed either a high-fat diet (60% kcal from fat) or a control diet (10% kcal from fat) for 8 weeks. Intraperitoneal glucose tolerance test (IPGTT) was performed at week 6. In the final week, animals received daily injections of tirzepatide (10 nmol/kg/day, intraperitoneal) or placebo (PBS). On the final day, animals were surgically implanted with catheters into the duodenum (for intraluminal lipid infusion) and the mesenteric lymph duct (for lymph collection). Body weight, energy intake, lymph flow rate, lymph triglyceride and, and lymph apolipoprotein B48 (ApoB48) were measured. Expression of key lipid metabolism genes in jejunal mucosa were assessed with RT-qPCR. Spatial transcriptomics were performed with 10X Genomics Visium on whole jejunum tissues. High-fat diet feeding increased intestinal lipid secretion compared with control diet. Short-term tirzepatide treatment attenuated intestinal lipid secretion in both dietary groups, along with modified expression of lipid metabolism genes. These findings support that tirzepatide suppresses lipid secretion from the gut, independent of dietary fat content or metabolic status. Spatial transcriptomics analyses are on-going.
Conclusions: These results suggest that GLP-1R/GIPR dual agonism with tirzepatide modulates lipid metabolism in the intestine to attenuate diet-induced intestinal lipid overproduction. GLP-1R/GIPR dual agonism with tirzepatide may represent a viable therapeutic strategy for managing dyslipidemia and reducing ASCVD risk.

Masters Student

Poster Presentations

P-1

Serum SOS1 as a prognostic biomarker and therapeutic target in progressive liver disease

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Background: Chronic liver disease follows a progressive course from fibrosis to cirrhosis and ultimately decompensation, driven by activation of hepatic stellate cells (HSCs). While downstream fibrogenic pathways such as transforming growth factor beta (TGFβ) signaling are well characterized, upstream molecular regulators remain incompletely defined, limiting biomarker discovery and therapeutic innovation. Son of Sevenless homolog 1 (SOS1), a guanine nucleotide exchange factor that activates rat sarcoma (RAS) signalling, integrates receptor tyrosine kinase inputs into extracellular signal-regulated kinase (ERK) and protein kinase B (AKT) cascades central to HSC activation. Although SOS1 inhibitors have entered early-phase oncology trials, their role in liver disease is unknown. We investigated whether SOS1 functions as a prognostic biomarker and tractable therapeutic target across progressive liver disease.
Hypothesis: We hypothesize that SOS1 acts as an upstream regulator of fibrogenesis, thereby driving progression from fibrosis to decompensation, and that its inhibition offers a tractable therapeutic strategy to improve survival in chronic liver disease.
Methods and Results: We prospectively studied a Nigerian cohort spanning fibrosis (n=12), cirrhosis (n=12), and decompensated cirrhosis (n=20). Serum SOS1 was quantified and related to fibrosis indices, biochemical markers, and 24-month survival using Cox regression and receiver operating characteristic (ROC) analysis. External validation was performed in The Cancer Genome Atlas–Liver Hepatocellular Carcinoma dataset (TCGA-LIHC). Mechanistic effects of pharmacological SOS1 inhibition (SOS-I, 10–20 nM) were tested in LX-2 stellate cells by extracellular matrix (ECM) staining, immunoblotting, and quantitative polymerase chain reaction (qPCR), and in vivo efficacy was evaluated in a carbon tetrachloride (CCl_4) plus acetaminophen murine model treated with SOS-I (150 mg/kg/day). Serum SOS1 levels increased progressively across fibrosis, cirrhosis, and decompensation ($p<0.001$). Elevated SOS1 predicted inferior 24-month survival (hazard ratio [HR] 1.75, 95% confidence interval [CI] 0.53–5.84) and correlated with the aspartate aminotransferase-to-platelet ratio index (APRI, r=0.75) and fibrosis-4 index (FIB-4, r=0.66). AUROC for advanced fibrosis discrimination was 0.70, complementing alpha-fetoprotein (AFP, 1.0) and fibronectin (FN1, 0.85). In TCGA-LIHC, SOS1 was consistently upregulated in tumors, outperformed AFP (AUC = 0.46) and matrix markers in diagnostic accuracy, and remained an independent prognostic factor after adjustment for age and sex (HR 1.50, $p=0.024$). In vitro, SOS-I suppressed fibronectin and collagen deposition, reduced alpha-smooth muscle actin (α-SMA), and downregulated FN1, collagen type I alpha 1 chain (COL1A1), and actin alpha 2 smooth muscle (ACTA2) transcripts, while restoring features of stellate cell quiescence. In vivo, SOS-I attenuated fibrosis, preserved liver architecture, reduced hepatocellular injury, lowered AFP and alanine aminotransferase (ALT), delayed tumorigenesis, and significantly prolonged survival ($p<0.05$).
Conclusions: SOS1 emerges as a novel biomarker and druggable regulator of liver disease progression. Its stepwise induction tracks with disease severity and survival, is validated externally in TCGA, and mechanistically links HSC activation to ECM accumulation. Pharmacological SOS1 blockade suppressed fibrogenesis and delayed hepatocarcinogenesis in vivo, highlighting translational potential. These findings position SOS1 as both a prognostic biomarker to refine patient stratification and a therapeutic target for antifibrotic intervention.

Postdoctoral Fellow

P-2

3D collagen hydrogel co-culture model to simulate cellular responses after vascular injury induced by percutaneous coronary intervention

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Background: Over 2 million individuals undergo percutaneous coronary intervention (PCI) each year. This procedure uses a stent to keep narrowed vessels open. Drug-eluting stents (DES), which release anti-proliferative agents like everolimus, are most commonly used to prevent pathological proliferation of smooth muscle cells (SMCs) after stent-induced vessel injury (restenosis). However, everolimus also inhibits the growth and migration of endothelial cells (ECs), which are essential for vascular wound healing and preventing restenosis. Most previous pre-clinical studies used cultured SMCs to screen anti-restenosis compounds. As a result, the side effects of everolimus are likely underestimated, and at least 10% of patients still develop in-stent restenosis after DES implantation. My study aims to develop a three-dimensional (3D) collagen hydrogel co-culture model to assess endothelial healing and EC/SMC proliferation after PCI-induced injury. The long-term goal is to use this co-culture model for advanced drug screening to improve treatment outcomes after PCI. My current study focuses on validating this model.
Hypothesis: The 3D co-culture collagen hydrogel model can simulate cellular responses after PCI injury.
Methods and Results: To test whether ECs will be activated in the hydrogel PCI model, as seen in patients after PCI. We embedded cell-traced human coronary artery SMCs in rat tail collagen type I (4mg/mL) and seeded a monolayer of human coronary artery ECs on top. We injured the endothelial layer and parts of the smooth muscle layer by scraping the gel surface with a P200 tip to create a 1 mm deep wound in the gel. Two adjacent lengthwise scratches were made for each gel. After 24 hours, we embedded the gel in optimal cutting temperature (OCT) blocks. Immunofluorescence staining of hydrogel sections showed increased ICAM-1 around scratched ECs compared to ECs far away from the scratch wound, confirming that our model can simulate endothelial activation, the immediate response to PCI injury.
To test whether SMCs will proliferate in our model, as seen in restenosis after PCI, injured and non-injured hydrogels were chased by EdU incorporation for 24 hours. Cells in the hydrogel were released by collagenase digestion and analyzed by flow cytometry to quantify the proportion of actively proliferating cells. Preliminary data showed an increase in the proliferation of both SMCs and ECs after endothelial injury, which indicates that the hydrogel can model restenosis in response to PCI injury.
Conclusions: The 3D hydrogel co-culture model is promising to simulate cellular responses after PCI injury, taking both ECs and SMCs into account for future drug screening.

PhD Student

P-3

Linking Gestational Diabetes to Future Heart Disease: The Role of Mitochondria and Protein Acetylation

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Background: Gestational diabetes mellitus (GDM) is the most common metabolic complication of pregnancy. In addition, studies reveal that intrauterine exposure to maternal GDM predisposes offspring to cardiomyopathy, insulin resistance, and altered cardiac structure and function. Yet, the mechanistic basis linking fetal GDM exposure to progressive cardiac dysfunction remains poorly defined.
Hypothesis: We hypothesized that fetal exposure to GDM disrupts offspring cardiomyocyte metabolic programming through impaired mitochondrial function and protein acetylation, thereby predisposing to cardiac hypertrophy and diastolic dysfunction across the life course. Furthermore, we proposed that modulation of SIRT3 expression would protect against these GDM-induced cardiometabolic impairments.
Methods: GDM was induced in rats and mice by feeding dams a high-fat, high-sucrose (HFS) diet before and during pregnancy and lactation, while control dams received a low-fat (LF) diet. Fetal rat ventricular cardiomyocytes (FRVC) were isolated at embryonic day 20 for U-^{13}C-glucose flux analysis, mitochondrial respiration, and calcium handling. Cardiac structure and function were tracked by echocardiography from late gestation through 12 months of age. Multi-omics profiling—including metabolomics, RNA-Seq, and mass spectrometry-based acetyl-proteomics—was performed on offspring hearts. To interrogate the role of mitochondrial protein acetylation, offspring of GDM dams were bred with cardiac-specific SIRT3 transgenic males and exposed postnatally to LF or HFS diets, followed by echocardiography and analyses of systemic metabolism.
Results: GDM-exposed offspring exhibited persistent left ventricular hypertrophy, increased posterior wall thickness, and impaired diastolic filling from fetal life through adulthood ($p<0.05$). In isolated FRVC, calcium flux and sarcoplasmic reticulum-dependent reuptake were increased (1.5–1.6-fold, $p<0.05$), while isoproterenol-stimulated glycolytic and TCA cycle fluxes were reduced. Metabolomic profiling revealed accumulation of long-chain acylcarnitines, particularly stearoylcarnitine, consistent with impaired mitochondrial oxidative metabolism. Transcriptomic analysis identified differential expression of genes regulating glucose transport and fatty acid oxidation (e.g., Irs2, Slc2a4, Pfkfb2, Pdk4, Cpt1a). At the protein level, GDM reduced SIRT3 expression in the heart and induced widespread hyperacetylation of mitochondrial proteins involved in fatty acid metabolism, the TCA cycle, and oxidative phosphorylation, including ATP5h, UQCRC1, SDHB, and MDH2. These effects were exacerbated by postnatal HFS diet exposure. Strikingly, cardiac-specific SIRT3 transgene expression prevented GDM- and diet-induced left ventricular hypertrophy, mitochondrial dysfunction, and glucose/insulin intolerance, with sex-specific patterns of protection observed.
Conclusions: This integrative, multi-omics analysis identifies mitochondrial oxidative metabolism and protein acetylation as key mechanisms linking fetal GDM exposure to long-term cardiac and metabolic dysfunction in the offspring. Stearoylcarnitine emerges as a novel biomarker of impaired mitochondrial metabolism, while SIRT3 functions as a critical protective factor against maladaptive mitochondrial acetylation and cardiometabolic disease. These findings establish mitochondrial protein acetylation as a developmental mechanism of intergenerational disease risk and support targeting SIRT3 for prevention of GDM-associated cardiovascular disease, with attention to sex-specific responses.

Other Research Staff

P-4

Mannose prevents atherosclerotic plaque development in diabetic ApoE KO mice by regulating liver genes involved in efferocytosis

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McGill University- Lady Davis Institute

Introduction: Type 2 diabetes is a major risk factor for cardiovascular diseases. Diabetic patients are more susceptible to atherosclerotic disease than non-diabetic individuals with comparable risk factors. This diabetes-induced atherosclerosis is primarily attributed to elevated blood glucose levels. Although moderating sugar intake is strongly recommended for diabetic patients, it remains challenging due to the numerous dietary sources of glucose. In contrast, D-mannose-a C-2 epimer of glucose plays a crucial role in protein glycosylation. We previously demonstrated that oral mannose supplementation exerts an atheroprotective effect and improves glucose tolerance in ApoE$^{-/-}$ mice fed a high-fat diet (HFD), without altering blood glucose or lipid levels.
Hypothesis: We hypothesize that mannose supplementation to HFD can delay or prevent atherosclerosis under diabetic conditions.
Methods and Results: Eight-week-old ApoE$^{-/-}$ mice were injected intraperitoneally with 50 mg/kg/day of the beta-cell cytotoxin streptozotocin (STZ) for five consecutive days to induce diabetes. Both diabetic and non-diabetic control mice were then fed a HFD supplemented with either 0% or 5% mannose for the following five weeks. At term, the body weight of mice injected with STZ was 23% less than that of controls ($P<0.05$), independent of mannose treatment. Mannose supplementation did not affect plasma lipid or glucose levels (non-diabetic: 103±13 [0%] vs 139±20 mg/dL [5%]; diabetic: 444±94 [0%] vs. 351±35 mg/dL [5%]). Atherosclerotic plaque burden, assessed via Oil Red O staining in the aortic sinus, was markedly increased in diabetic mice receiving 0% mannose compared to non-diabetic controls (1.3±0.03 mm² vs. 0.34±0.07 mm²). Notably, this increase was completely prevented in diabetic mice supplemented with 5% mannose (0.4±0.1 mm² vs. 1.3±0.03 mm²). These findings suggest that mannose supplementation prevents diabetes-associated atherosclerosis without altering blood glucose levels. No significant differences in plaque composition of Mac-2, CD68, or α-smooth muscle actin were noted in diabetic mice receiving 0 or 5% mannose. Finally, diabetic mice exhibited a significant 1.8-fold increase in liver-to-body weight ratio, which was prevented by mannose supplementation (0.096±0.01 vs. 0.070±0.004). This was accompanied by a two-fold increase in hepatic expression of ABCA-1 (a mediator of cholesterol efflux) and c-Mer tyrosine kinase (Mertk, a mediator of efferocytosis) in diabetic mice treated with mannose ($P<0.05$).
Conclusions: These results indicate that oral mannose supplementation to HFD prevents the development of atherosclerosis in diabetic ApoE-/- mice. This protective effect may be mediated through the regulation of hepatic genes involved in efferocytosis and cholesterol efflux.

Other Research Staff

P-5

Hepatic Monooxygenase X regulates systemic metabolism and energy expenditure

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Background: Monooxygenase X (MOX/Moxd1) is a part of the copper (II) ascorbate-dependent monooxygenase family. Elevated MOX expression has been reported in various liver diseases, including metabolic dysfunction-associated steatotic liver disease (MASLD) and alcohol-related liver disease (ALD). Despite these associations, the physiological substrates and/or products of MOX remain elusive, and the precise role of MOX in liver physiology and pathophysiology is poorly understood. Notably, a knockout of Moxd1 in mice has never been studied before.
Hypothesis: Given MOX is reported to be involved in various metabolic diseases of the liver, we hypothesize that MOX may play a regulatory role in energy metabolism.
Methods and results: To address these knowledge gaps, we generated mice with a hepatocyte-specific deletion of the Moxd1 gene (Moxd1-ΔHep) using the Cre-lox recombination system by crossing Moxd1-Flox mice with mice expressing a Cre-recombinase driven by the albumin promoter. We then performed a series of standard metabolic assessments to investigate how hepatocyte-specific knockout of Moxd1 influences whole-body energy metabolism. On a standard chow-diet, Moxd1-ΔHep mice were generally indistinguishable from Moxd1-Flox control mice. A body-composition analysis using EchoMRI revealed a modest reduction in fat mass in Moxd1-ΔHep mice (Student's t-test, $p<0.05$), with no overt changes to body weight, naso-anal length, or lean mass. Liver histology (H&E) and picrosirius red (PSR) showed no overt alterations to liver microanatomy or collagen accumulation. Interestingly, Moxd1-ΔHep mice had lower fasting blood glucose after a 6-hour fast ($p<0.05$), but not after a 16-hour prolonged fast. During the intraperitoneal glucose tolerance test (IP-GTT), Moxd1-ΔHep mice demonstrated improved glucose clearance (iAUC analysis, $p<0.05$). Insulin sensitivity assessed by the insulin tolerance test (ITT) did not show any significant differences (iAOC analysis), however, partly confounded by reduced basal blood glucose levels in Moxd1-ΔHep mice. While plasma total cholesterol, triglycerides, and non-esterified fatty acids were not significantly changed between Moxd1-Flox and Moxd1-ΔHep mice, hepatic cholesteryl esters and triglycerides were significantly reduced ($p<0.05$). Consistently, there was reduced oil red O staining in Moxd1-ΔHep mice ($p<0.05$). To further characterize these metabolic alterations, 10-week-old male Moxd1-Flox and Moxd1-ΔHep mice were assessed in metabolic cages. While food intake, water intake, activity, and respiratory exchange ratio (RER) was similar between groups, both oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were significantly increased during the light cycles ($p<0.05$), indicating increased metabolic rate independent of changes to fuel utilization. Consistently, energy expenditure was elevated in Moxd1-ΔHep mice during the light cycle (adjusted for body weight with ANCOVA analysis, $p<0.05$). All mice used in these studies were male.
Conclusions: This preliminary data suggests that hepatic MOX deficiency alters whole-body energy metabolism, possibly involving the regulation of hepatic lipid stores, glucose handling, and energy expenditure. Ongoing studies aim to elucidate the underlying mechanisms driving these observations.

PhD Student

P-6

The Role of S1PR1 Signaling in Vascular Smooth Muscle Cell Cholesterol Metabolism and Atherosclerosis

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Background: Atherosclerosis, a major cause of global mortality, is a chronic disease in which fatty deposits and cells build up as plaques in the arterial walls, which may rupture and lead to restricted blood flow, thereby causing adverse cardiovascular events. Atherosclerosis involves chronic inflammation within the arterial wall, triggering immune responses and vascular smooth muscle cell (VSMC) proliferation and recruitment. VSMCs can adopt a proliferative and migratory phenotype, accumulate lipids, and contribute to the inward thickening of the artery wall. Lineage-tracing studies have reported that smooth muscle-derived cells constitute approximately 50% of plaque cells. Reverse cholesterol transport, mediated by the ATP-binding cassette transporters ABCA1 and ABCG1, plays a key role in maintaining cholesterol homeostasis in arterial wall cells, thereby reducing plaque progression.
Hypothesis: We hypothesize that sphingosine-1-phosphate receptor 1 (S1PR1) signaling in VSMCs promotes atherosclerosis by suppressing ABCA1-mediated cholesterol efflux, leading to increased lipid accumulation and foam cell development.
Methods and Results: We found that VSMC-specific knockout of S1PR1 reduces lipid accumulation in vitro when VSMCs are cultured in media containing cholesterol complexed with methyl-β-cyclodextrin, which forces cholesterol uptake. Conversely, pharmacological activation of S1PR1 with SEW2871 significantly increased lipid accumulation in murine VSMCs. Preliminary Western blot and RT-PCR data have shown reduced expression of ABCA1 under SEW2871-induced S1PR1 stimulation. RT-PCR also revealed decreased mRNA levels of RXR-α/β and LXR-α/β, transcription factors that promote ABCA1 expression, in SEW2871-treated VSMCs. In vivo, VSMC-specific knockout of S1PR1 resulted in reduced aortic sinus plaque burden in ApoE knockout mice fed an atherogenic diet.
Conclusions: These findings suggest that S1PR1 signaling in VSMCs may exacerbate atherosclerosis by inhibiting cholesterol efflux pathways, thereby promoting lipid accumulation and foam cell development. Future studies will assess the dependency of ABCA1 regulation on S1PR1 signaling, in vitro, and determine whether impaired cholesterol efflux can be rescued or even improved with the S1PR1 inhibitor Ex26.

PhD Student

P-7

Direct Glucagon Injection into the Nucleus Tractus Solitarius Lowers Food Intake in Non-Diabetic and Diabetic Rats

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Background: Imbalances in energy and glucose metabolism underlie the growing prevalence of obesity and diabetes. Thus, there is a demand for therapies that promote satiety, stimulate caloric expenditure, and improve insulin sensitivity. Glucagon regulates metabolism and has beneficial actions on weight management and metabolic health, despite its hyperglycemic effects, particularly when used in combination with GLP-1 receptor agonists. However, its mechanisms are not clearly elucidated, particularly in the nucleus tractus solitarius (NTS) – a key hindbrain area that controls metabolism.
Hypothesis: We aimed to determine whether acute NTS glucagon affects metabolic energy balance in non-diabetic and type 2 diabetic (T2D) rats and hypothesize that glucagon action, selectively in the NTS, reduces food intake and increases energy expenditure.
Methods and Results: Eight-week-old male Sprague Dawley rats underwent NTS cannulation surgery. To explore the effects of NTS glucagon on energy balance, a subset of non-diabetic animals was assessed using metabolic cages for 48h after receiving an acute injection of glucagon or vehicle (control) into the NTS. Whereas there was no effect on activity, energy expenditure, or respiratory exchange ratio, NTS glucagon significantly reduced food intake compared to vehicle controls, leading to a reduced total energy balance. To assess the effect of acute NTS glucagon in response to a hunger stimulus, non-diabetic rats were fasted overnight and refed ad libitum the following day after receiving an injection of glucagon, glucagon receptor antagonist (GcgrA), GcgrA+glucagon co-injection, or vehicle into the NTS, and caloric intake was measured for 6h. NTS glucagon reduced caloric intake in non-diabetic rats. Whereas NTS GcgrA alone did not affect caloric intake, it potently blocked the ability of glucagon to lower caloric intake, demonstrating the requirement of the glucagon receptor to mediate the hypophagic effect of glucagon. Western blot showed an increase in pCREB, a downstream target of canonical glucagon-receptor signalling, following NTS glucagon injections compared to NTS vehicle control. A subset of rats was rendered T2D by intraperitoneal injections of nicotinamide and streptozotocin, followed by high-fat diet feeding, and underwent the same fasting-refeeding protocol. In T2D rats, NTS glucagon also lowered caloric intake compared to saline controls. Direct activation of PKA, an established mediator of glucagon receptor signalling, within the NTS using Sp-cAMPs likewise reduced caloric intake to a similar extent as NTS glucagon in T2D rats. To assess whether the effects of NTS treatments persisted over time, food intake and body weight were also measured 24h and 48h after NTS administration. Importantly, rats that received NTS glucagon and Sp-cAMPs did not experience a "rebound" in food intake or body weight in this timeframe after acute caloric reduction.
Conclusions: We reveal a novel target for glucagon action within the NTS to regulate caloric intake. These findings support glucagon's anorectic effect through an NTS glucagon receptor signalling pathway. Understanding a mechanism of NTS glucagon to regulate food intake may help in the development of therapeutics to combat obesity-related metabolic diseases; notably, GLP-1-/glucagon receptor co-agonists are being developed to be a more potent therapy for body weight management and metabolic health.

Other Research Staff

P-8

Loss of PITX2C triggers cardiac inflammation and mitochondrial dysfunction in arrhythmias

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Background: Atrial fibrillation (AF) is the most common cardiac arrhythmia, and patients with AF are faced with increased morbidity and mortality. The most significant genetic risk factor associated with AF susceptibility is the paired-like homeodomain transcription factor 2 (PITX2) gene. PITX2C regulates transcription of key cardiac rhythm genes; loss of PITX2C results in maladaptation in atrial structure, Ca2+ handling, and electrophysiology that is the fundamental basis of arrhythmogenesis. However, how metabolic remodelling acts as a primary driver of arrhythmias has been of recent interest. Evidence suggests that PITX2C is a direct transcriptional regulator of key metabolic genes, yet the downstream mechanisms remain elusive. In addition, how cardiac inflammation acts upstream of metabolic remodelling in the loss of PITX2C has not been explored.
Hypothesis: Decreased PITX2C function will activate inflammatory pathways, impairing mitochondrial function to elevate oxidative stress which fosters a pro-arrhythmic environment.
Methods: Neonatal rat atrial myocytes (NRAMs) transfected with Pitx2c siRNA were leveraged to assess the acute effects of the loss of PITX2C. Bulk RNA-seq of Pitx2c knockdown NRAMs was performed and gene set enrichment analysis identified affected pathways (FDR>0.25). Cardiomyocyte function was assessed by characterizing readouts of excitation-contraction coupling including intracellular Ca2+ handling using Fluo-8, sarcomere assembly through immunocytochemistry, and mitochondrial function through Seahorse assays. As metabolic dysfunction gives rise to oxidative stress, mitochondrial derived reactive oxygen species (mROS) were quantified using MitoSOX. To examine how redox imbalance directly contributes to cardiac dysfunction, an antioxidant, N-acetyl cysteine (NAC) was administered and cardiomyocyte function was assessed thereafter.
Results: Pathway analysis identified dysregulated inflammatory pathways, suggestive of a role of PITX2C in an immune-related transcriptional response. In addition, downregulation of glycolysis and oxidative phosphorylation was observed. Seahorse assays assessing oxidative phosphorylation suggest decreased maximal respiration in response to FCCP, a potent mitochondrial uncoupler which increases the permeability of the mitochondrial membrane to protons. The compensatory glycolytic capacity was also impaired in Pitx2c knockdown NRAMs. This indicates a clear role of PITX2C in regulating atrial bioenergetics yet the link between the inflammatory response and mitochondrial health and function remains elusive. However, a proposed mechanism is through inflammatory signaling which impairs mitochondrial health and function that elevates oxidative stress. Indeed, we have observed an increase in mROS in Pitx2c knockdown NRAMs. Downstream redox sensitive cellular pathways were then assessed. We have observed that Pitx2c knockdown NRAMs display decreased contractile function owing to an increased frequency of abnormal oscillations, highlighting early (EADs) and delayed (DADs) afterdepolarizations, a pro-arrhythmic mechanism. In further detriment of contractility, we have observed mis-localization of Titin, an integral protein to the sarcomeres. Interestingly, boosting the antioxidant response with NAC restores Ca2+ cycling where EADs and DADs are reduced in Pitx2c knockdown NRAMs and Titin localization to the sarcomeres is restored.
Conclusions: PITX2C maintains cardiac bioenergetic and redox balance; the loss of PITX2C results in dysregulation of metabolic pathways including glycolysis and oxidative phosphorylation and an elevation in oxidative stress that ramifies cardiomyocyte dysfunction. Future studies will determine how PITX2C couples the inflammatory responses and cardiac metabolism which drives AF pathogenesis.

Masters Student

P-9

Targeting Lymphatic Endothelial Cells via APOA1-Loaded Platelet-Derived Extracellular Vesicles

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Background: The lymphatic system plays a crucial role in the clearance of peripheral waste. Dysfunctions in this system contributes to the development of several diseases, such as atherosclerosis. Our team has shown that impaired lymphatic vessel (LV) contraction precedes atherogenesis, thereby ultimately compromising cholesterol and inflammation clearance from blood vessel walls. One approach to therapeutically improving lymphatic function is to harness endogenous mechanisms that maintain lymphatic endothelial cell integrity. Platelets play a key role in preserving lymphatic function through their interactions with lymphatic endothelial cells (LECs) at blood-lymph interfaces. We have demonstrated that treatment with apolipoprotein A-1 (APOA1) enhances this protective mechanism in atherosclerotic mice by increasing platelet adhesion to LECs. This newly identified pleiotropic role for APOA1 leads to an enhancement in the integrity of LECs and LV function, while simultaneously limiting atherosclerosis progression. However, considering that lymph is devoid of platelets and that exogenous APOA1 has limited access to the lymph, their effects within the lumen of LVs are suboptimal. Nonetheless, platelets can extend their benefits in the lymph through the release of extracellular vesicles (EVs) upon activation/adhesion. We have recently published that these platelet-derived extracellular vesicles (PEVs) are capable of improving both LEC integrity and lymphatic contractility. Our preliminary results also suggest that APOA1 acts synergistically with PEVs to further enhance LEC integrity.
Hypothesis: To overcome the poor lymphatic accessibility of exogenous APOA1, we propose loading PEVs with APOA1 to enable targeted delivery to LECs, where APOA1 could, in turn, enhance PEV uptake.
Methods and Results: Electroporation has proven effective for incorporating APOA1 within PEVs, without compromising their stability. The loading does not alter how PEVs are internalized by cells, which occurs primarily through clathrin-dependent endocytosis, with most PEVs internalized after 24 hours.
Conclusions: Ultimately, this innovative approach could more effectively target the lymph to correct the lymphatic dysfunction observed in atherosclerosis, and thereby slow its progression.

PhD Student

P-10

RIPK1 inhibitors (Nec-1s and GSK'772) decrease cell death in TNF-a and ZVAD-fmk-induced necroptosis in peritoneal macrophages from C57BL6 and PDZK1 KO mice.

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Background: PDZ domain containing 1 (PDZK1) is a cytosolic adaptor protein with 4 protein-protein interacting PDZ domains. In macrophages, PDZK1 mediates signaling in response to HDL binding to SR-B1, leading to activation of PI3K/Akt signaling, inactivation of TAK1 and TBK1, and suppression of RIPK1, RIPK3, and MLKL-mediated necroptosis, characterized by membrane pore formation. Necrostatin-1s (Nec-1s) is a well-studied RIPK1 inhibitor, known to reduce necroptosis in atherosclerotic plaques in aortic sinus regions. GSK2982772 (GSK'772) is a clinically relevant RIPK1 inhibitor that inhibits RIPK1-dependent inflammation but has yet to be assessed on necroptosis and coronary disease. Necroptosis can be activated using TNFα and pan-caspase 8 inhibitor, ZVAD-fmk in macrophages.
Hypothesis: GSK'772 and Nec-1s protect against TNF-a and ZVAD-fmk induced necroptotic cell death in a concentration dependent manner.
Methods: Wild-type (WT) and PDZK1 KO mice fed a normal chow diet, were injected intraperitoneally with 10% thioglycolate to recruit macrophages to the peritoneal cavity (MPMs). Four days later, macrophages were flushed from the peritoneal cavity with PBS containing 5mM EDTA. Cells were washed and then cultured for 24 hours in DMEM with 10% Fetal Bovine Serum, L-Glutamine, Penicillin, and Streptomycin. The cells were then cultured for 24 hours in media containing 3% fetal bovine lipoprotein deficient serum. Following this, cells were treated for 24 hours with 50 ng/mL murine TNFα and 50 μM ZVAD, to induce necroptosis, either in the absence or presence of varying concentrations of either Nec-1s or GSK'772 (0 to 10 μM). Cell death was measured by release of cytosolic lactate dehydrogenase (LDH) into the cell culture medium. Propidium iodide (PI) staining was performed on unfixed cells then cells were fixed and stained with DAPI and analyzed by fluorescence microscopy to determine percentage of cells with disrupted plasma membranes as determined by the proportion of DAPI stained nuclei (total) that were also positive for PI. In separate experiments, cells were also treated with varying concentrations of mTNFα (0 to 100 ng/ml) in the presence of 50 μM ZVAD and cell death was measured with LDH assay.
Results: WT and PDZK1 KO MPMs showed an increase in measured cell death with increasing TNFα dosing and a reduction in cell death with increased dosing of either GSK'772 or Nec-1s. Results of PI staining mirrored results of LDH release.
Conclusions: GSK'772 and Nec-1s reduce cell death in TNFα and ZVAD-induced necroptosis in both WT and PDZK1 KO MPM's. We have previously reported increased necroptosis, necrotic core size and atherosclerotic plaque sizes in PDZK1-deficient compared with PDZK1 expressing LDLR KO mice fed a high fat, Western type diet. We will explore if treating PDZK1 deficient LDLR KO mice with GSK'772 reduces diet induced atherosclerosis and plaque necroptosis.

Masters Student

P-11

Characterization of Novel Mutations in the LDLR identified in Children with Familial Hypercholesterolemia

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Background: Familial Hypercholesterolemia (FH) is an autosomal genetic disorder caused by mutations in genes that regulate the metabolism of low-density lipoprotein cholesterol (LDL-C). The majority of mutations associated with FH occur in the LDL receptor (LDLR) gene. Currently, there are no effective medications available to lower LDL-C levels in patients with homozygous or combined heterozygous FH, underscoring the urgent need for novel lipid-lowering.
Hypothesis: Two patients diagnosed with FH were admitted to our hospital. Patient A exhibited elevated total cholesterol levels and decreased urinary amylase levels. Cardiac ultrasound revealed mild aortic valve stenosis and aortic valve regurgitation. A combination of statins and ezetimibe significantly reduced LDL-C levels in this patient. In contrast, the same combination of statins and ezetimibe was ineffective in lowering LDL-C levels in Patient B. Based on the Whole-exome sequencing, this study hypothesizes that the mutations of patients caused conformational change, thereby affected the stability of the LDLR protein, and lead to varying clinical treatment outcomes.
Methods and Results: Whole-exome sequencing was employed to identify mutations in the LDLR gene in patients with FH. Site-directed mutagenesis was used to introduce these identified mutations into the wild-type LDLR. Transient transfections were performed using Lipofectamine 3000 to express both the wild-type and mutant LDLR in cultured human hepatocytes, specifically Huh7 cells. The effect of these mutations on the spatial conformations of LDLR was assessed by modeling its three-dimensional structure with PyMOL software. From our findings, patient A had a combination of heterozygous variants in the LDLR gene, specifically c.128A>G (K43R) and the deletion of exons 16-18. Patient B was diagnosed with homozygous FH and had a variant in the LDLR gene, c.663(exon 4)-c.683(exon 4)dup (D221-D227 duplication). The mutations c.128A>G and c.663_683dup were novel findings in the LDLR gene. Three-dimensional structure modeling indicated that all three mutations caused conformational changes in the LDLR. However, Western blot analysis revealed that K43R and D221-D227 duplication in LDLR did not significantly affect the expression of the receptor in Huh7 cells.
Conclusions: While we observed differing responses to standard treatments between the two patients, mutations K43R and D221-D227 dup in the LDLR gene do not appear to significantly impact the stability of the LDLR protein. Further studies, including Dil-LDL uptake and binding assays with PCSK9, are planned to explore how these mutations impair LDLR function and to assess whether the mutated receptors retain any functionality through rescue experiments.

PhD Student

P-12

Protective Effects of the loss-of-function PCSK9-Q155H Variant on Lipid Regulation and Fatty Liver Disease Progression

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Background: Proprotein convertase subtilisin/kexin type 9 (PCSK9) plays a central role in regulating circulating atherogenic lipids by promoting the degradation of cell surface low-density lipoprotein receptors (LDLR), making it a major target for lipid-lowering therapies. Emerging evidence also implicates PCSK9 in cellular stress pathways, including the regulation of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR). Lebeau et al. (2021) reported that ER retention of a naturally occurring loss-of-function (LOF) PCSK9-Q152H variant in its unprocessed zymogen state does not increase ER stress or activate the UPR. Unexpectedly, this variant elevates protein abundance of key ER-resident chaperones such as glucose-regulated protein 78-kDa (GRP78) and 94-kDa (GRP94) in both cell culture and mouse models of liver-specific overexpression. Human carriers of this variant exhibit a ~79% reduction in circulating PCSK9 levels and significantly lower total and LDL cholesterol compared to non-carriers and carriers of gain-of-function variants. Notably, these individuals show no evidence of cardiometabolic disease or liver injury, even in older and very old adults.
Hypothesis: We hypothesize that the PCSK9-Q155H LOF variant modulates systemic and hepatic lipid regulation, thereby influencing susceptibility to MAFLD/MASH and its progression to fibrosis and inflammation.
Methods: Given that inhibition of PCSK9 secretion and ER retention in its zymogen state has been linked to cardiometabolic protection, we generated a whole-body CRISPR knock-in mouse model of this variant, expressing endogenous levels of PCSK9-Q155H. Littermate control cohorts were bred and randomized to either a high-fat high-fructose (HFHFr) diet or a chow diet control group.
Results: Homozygous and heterozygous mice carrying the LOF variant displayed lower circulating atherogenic lipids and total cholesterol compared to wild-type littermates, along with a modest but significant reduction in circulating PCSK9 levels.
Conclusions: These findings support a protective role of the PCSK9-Q155H variant in lipid regulation and highlight its potential relevance in mitigating progression of fatty liver disease.

PhD Student

P-13

Estrogens Differentially Modulate Lymphatic Function and Atherosclerosis in Menopause-Modeled Female Mice and Age-Matched Males

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Background: Despite major advances in risk factor management, coronary artery disease remains the most frequent cause of death in Western countries. Atherosclerosis, a cholesterol-driven inflammatory condition, is the predominant cause of cardiovascular disease (CVD). The lymphatic system is a prerequisite for the removal of cholesterol from the arterial wall. Subsequently, we reported that a dysfunction in collecting lymphatic vessels occurs prior to the onset of atherosclerosis in LDL receptor-deficient (Ldlr-/-) mice. Moreover, we have demonstrated that early stimulation of the lymphatic system with selective vascular endothelial growth factor-C (VEGF-C 152s) before the administration of a pro-atherosclerotic regimen in Ldlr-/- mice, improved lymphatic vessel capacities while limiting plaque progression. Hormonal changes throughout life constitute critical determinants of cardiovascular vulnerability.
Hypothesis: Since the role of the lymphatic system in CVD has only recently been recognized, no studies have yet addressed how hormonal changes influence lymphatic transport in individuals predisposed to CVD. This study aims to investigate sex differences in the effects of 17β-estradiol (E2) treatment on lymphatic function and atherosclerotic burden.
Methods and results: We modeled menopause in atherosclerosis-prone $Ldlr^{-/-}$ female mice through ovariectomy. These mice, along with age-matched males, were treated with E2 to assess the impact of hormone therapy on in vivo lymphatic function and atherosclerosis. E2 reduced lesion burden and improved lymphatic transport in $Ldlr^{-/-}$ male mice through non-genomic pathways. However, it worsened the lymphatic function of ovariectomized female mice in an ERα-dependent mechanism. Enhancing lymphatic transport via prior VEGF-C stimulation beforehand prevented this effect and reduced atherosclerotic plaque formation.
Conclusions: Taken together, our results highlight the complexity of how E2 therapy may differentially influence the lymphatic system and cardiovascular health, paving the way for more effective targeted interventions and novel therapeutic strategies in high-risk populations undergoing hormonal transitions.

PhD Student

P-14

Mannose reduces atherosclerosis by regulating bone marrow myeloid cell maturation, glycolytic and mitochondrial function.

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Background: Excessive sugar intake is a well-established contributor to metabolic disorders and cardiovascular diseases, including atherosclerosis. While glucose dominates the nutritional landscape, there are alternative sugars such as D-mannose, a C-2 epimer of glucose, with unique metabolic properties. Mannose interferes with glycolysis by competing with glucose and has been shown to reduce mitochondrial reactive oxygen species and pro inflammatory cytokine production in macrophages.
Hypothesis: We hypothesized that D-mannose supplementation to high fat diet (HFD) regulates immune cell metabolic function, thereby attenuating atherosclerosis.
Methods and Results: Male ApoE-/- mice were fed a high fat diet (HFD) for 9 weeks. Concurrently, they received tap water containing 0 or 20% D-mannose. Total body weight and lipid levels were similar in both groups. OilRedO staining of the aortic sinus revealed a significant reduction in atherosclerotic plaque size in mannose-treated animals (0.39±0.06 mm^2) vs controls (0.69±0.06 mm^2). Concurrently, levels of circulating Ly6CHI monocytes and neutrophils, which are elevated by HFD, were normalized by mannose treatment. Since these cells are mobilized from the bone marrow (BM), we evaluated BM-derived myeloid cell development, maturation, and metabolism. Flow cytometry demonstrated that $cKit^+Sca1^-$ myeloid progenitor cells were reduced in 20% mannose mice (7.15±1.08%) compared with 0% control mice (11.54±0.75%). Additionally, the ratio of immature/mature Ly6G+ neutrophils, determined by CXCR2 expression, was decreased in mice treated with mannose (0.7±0.1 vs 1.4±0.2), suggesting an accelerated maturation of BM neutrophils. Mitochondrial function and glycolysis are known to be accentuated in atherosclerosis. Interestingly, BM cell mitochondrial superoxide level, assessed by MitoSOX staining, was significantly lower in the mannose group (3.89±0.4%) than controls (7.34±1.0%). This finding was supported by Seahorse oxygen consumption rate (OCR) data, showing a 23% decrease in spare respiratory capacity in BM cells from mice receiving 20% mannose (129 ± 45 pmol/min) compared with 0% mannose (166 ± 18 pmol/min). Furthermore, glycolytic capacity was also reduced in BM cells of 20% mannose-treated mice (28±5 mpH/min) compared with controls (58±7 mpH/min), as indicated by the extracellular acidification rate (ECAR) assay. In vitro stimulation of naïve myeloid cells with the plasma of 20% mannose-treated mice also reduced ECAR compared with 0% mannose plasma. These data suggest that mannose normalizes metabolic activity of BM cells under pro inflammatory conditions.
Conclusions: Atherosclerosis is associated with altered numbers and metabolic function of myeloid-derived cells. We show that in ApoE-/- mice fed a HFD, oral mannose supplementation regulates BM progenitors and myeloid cell maturation and abates their glycolytic activity and mitochondrial function. These effects may be responsible of the athero-protective effect of mannose in ApoE-/- mice.

PhD Student

P-15

PITX2C Deficiency Drives Atrial Metabolic Remodeling and Redox Imbalance in a Zebrafish Model of Cardiac Arrhythmia

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Background: Genetics is a pivotal risk factor for atrial fibrillation (AF), the most prevalent cardiac rhythm disorder. A non-coding region on chromosome 4q25, upstream of the gene encoding the transcription factor PITX2C, is strongly associated with AF. Recent data have suggested that PITX2C regulates cardiomyocyte metabolism and the antioxidant response to stress. However, the specific downstream metabolic alterations remain unclear.
Hypothesis: PITX2C deficiency disrupts cardiac metabolic homeostasis and redox balance, leading to atrial-specific metabolic remodeling and oxidative stress that contribute to AF pathogenesis.
Methods and Results: Using a pitx2c-deficient zebrafish model that recapitulates AF-like phenotypes, we analyzed metabolic and redox changes. Transcript analysis revealed atrial-specific up-regulation of gapdh (glycolysis) and down-regulation of ascl1b (lipid metabolism), with no corresponding ventricular changes. Targeted metabolomics of 97 central-carbon metabolites by UPLC–MRM/MS showed atrial-specific decreases in glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, and mannose-6-phosphate in pitx2c-/- hearts. Pathway enrichment highlighted glycolysis/gluconeogenesis and sugar metabolism as major disrupted nodes. A trend toward a higher $NADP^+$/NADPH ratio indicated increased oxidative demand, suggesting redox imbalance. Consistent with this, direct measurements of oxidative stress using electron paramagnetic resonance (EPR) spectroscopy demonstrated significantly elevated superoxide levels in both juvenile and adult pitx2c mutant hearts, confirming early-onset oxidative stress. Oil Red O staining further revealed significant lipid accumulation in adult but not young-adult pitx2c-/- hearts, indicating lipid deposition arises secondarily during disease progression. Importantly, overexpression of endogenous antioxidant genes (sod1, sod2) in mutant larvae improved heart rate variability, demonstrating that enhancing antioxidant defenses can mitigate arrhythmic phenotypes.
Conclusions: In conclusion, PITX2C deficiency induces atrial-specific remodeling of carbohydrate and lipid metabolism together with redox imbalance, contributing to cardiac dysfunction in AF. These findings link genetic susceptibility at PITX2C to metabolic vulnerability in the atrium and suggest antioxidant modulation as a potential therapeutic strategy.

PhD Student

P-16
Metabolic consequences of ovariectomy and sleep fragmentation

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Background: Women with type 2 diabetes mellitus (T2DM) have a 3–7-fold higher cardiovascular disease (CVD) risk compared to just a 2–3-fold increased risk in men with T2DM. During perimenopause, hormonal shifts lead to multiple adverse health outcomes, including central obesity, insulin resistance, dyslipidemia, inflammation, diastolic cardiac dysfunction, and sleep disruption. Therefore, menopause is a major sex-specific CVD risk factor.
Hypothesis: We hypothesize that menopause promotes hyperglycemia and increases body weight in sleep disruption.
Methods: Female mice underwent an ovariectomy (OVX) surgery to simulate menopause and were compared to sham-operated females, intact females, and intact males. All mice were fed chow for two weeks, and then assessed by EchoMRI and blood sampling before entering two weeks of sleep fragmentation, after which EchoMRI, blood collection, and an OGTT were performed. At week 2, they were transferred to a high-fat high-cholesterol diet for four weeks to induce obesity and insulin resistance, followed by a second round of sleep fragmentation with repeated measurements for EchoMRI, blood collection and OGTT, while body weight was monitored weekly.
Results: Preliminary results show that at week 2, OVX females showed elevated glycemia during OGTT despite no differences in body weight compared to intact and sham females. By week 6, OVX females displayed greater body weight and fat mass than intact females, with no change in lean mass, and also exhibited higher circulating lymphocyte count than the other groups. A second OGTT at week 8 revealed continued elevated glucose excursion in OVX females relative to intact and sham females, although all groups increased with the high-fat high-cholesterol diet feeding. Consistent with increased body weight, our preliminary results reveal that OVX females had larger livers as well as a higher amount of epididymal, mesenteric, retroperitoneal, and inguinal white adipose tissue compared to intact females.
Conclusions: After confirming the metabolic effects of OVX and high-fat diet under sleep disruption, we will next isolate the contribution of sleep fragmentation to metabolic dysregulation.

Other Research Staff

P-17
Monounsaturated Fats Protect Against Cholesterol Crystals in Cultured Liver Cells

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University of Saskatchewan

Background: Metabolic dysfunction associated steatotic liver disease (MASLD) is a condition in which fat accumulates in the liver. By itself, this is relatively benign, but MASLD can progress to the more severe state of metabolic dysfunction associated steatohepatitis (MASH). MASH develops when the liver acquires pathological inflammation. Therapies are urgently needed for MASH, as it is now a leading cause of liver cancer and failure. Progression of MASLD to MASH is due, in part, to excess storage of cholesterol, which then precipitates into crystals in lipid droplets. The mechanism by which cholesterol forms crystals in lipid droplets is unclear, but this event is thought to trigger liver inflammation. Understanding this mechanism may reveal how to block crystal formation in the liver as a therapeutic solution for MASH.
Hypothesis: Our lab recently discovered that sterol O-acyltransferase 1 (SOAT1) inhibition blocks crystal formation in hepatocytes, suggesting that liver crystals are composed of cholesteryl ester. However, it is unclear how fatty acid saturation influences cholesterol crystal properties and formation. We hypothesize that monounsaturated fatty acids will reduce cholesterol crystallization in hepatocytes.
Methods: Hep3B cells were incubated for 48 hours following cholesterol loading and treatment with palmitic acid, oleic acid or palmitoleic acid. At the endpoint, cells were stained with fluorescent dyes Bodipy and Hoechst to visualize lipid droplets and nuclei, respectively. The cholesterol crystals were detected by their birefringent property under polarized light. Experiments were done in 4 independent replicates. Statistical analysis was conducted using one-way ANOVA with Tukey's post hoc test for multiple comparisons (GraphPad Prism), with $p < 0.05$ considered significant.
Results: Both oleic acid and palmitoleic acid significantly reduced cholesterol crystal formation, whereas palmitic acid, a saturated fatty acid, did not. Monounsaturated fatty acid treatment was associated with a marked increase in lipid droplet size, area, and number compared with non-monounsaturated fatty acid conditions. Notably, this expansion of lipid droplets inversely correlated with cholesterol crystal burden, suggesting that monounsaturated fatty acids might promote cholesterol solubilization within the lipid droplet and thereby prevent crystal formation.
Conclusions: Both oleic acid and palmitoleic acid protect hepatocytes from cholesterol crystals while promoting lipid droplet accumulation. These findings support the idea that fatty acid saturation can modulate hepatic cholesterol crystallization. This provides a mechanistic basis for the hepatoprotective effects of monounsaturated fatty acid-enriched diets and highlights lipid droplet remodelling as a potential therapeutic strategy to mitigate cholesterol crystal-driven liver injury.

Other Research Staff

P-18

Fourier-transformed Infrared Spectromicroscopy reveal Cholesteryl Esters, Not Free Cholesterol, Predominate in Hepatic Crystals of Diet-Induced MASH

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Background: Metabolic dysfunction–associated steatohepatitis (MASH) develops under conditions of sustained metabolic imbalance and dietary excess, leading to liver damage. A striking feature is the emergence of cholesterol crystals within hepatocyte lipid droplets, reflecting disrupted lipid storage and serving as a potential trigger of liver inflammation. Although these crystals can be visualized under polarized light microscopy due to their birefringence, conventional histology provides little insight into their composition. Distinguishing between free cholesterol, cholesteryl esters, phospholipids, and triglycerides is important as these lipid classes differ in solubility, physical properties, and pathological contribution. Fourier-transform infrared (FT-IR) spectromicroscopy offers a non-destructive, label-free method to probe liver tissue composition, and generating spectral maps that distinguish biomolecules such as lipids, proteins, and carbohydrates without perturbing the sample.

Hypothesis: The biochemical nature of cholesterol crystal–bearing regions in MASH remain poorly defined. Here, we use FT-IR imaging to test the hypothesis that mouse liver tissue containing birefringent crystals have compositional signatures indicative of specific lipid classes, with cholesteryl esters representing a dominant component of these deposits.

Methods: Liver samples were collected from mice fed MASH-inducing high-fat, high-fructose, high-cholesterol (HFFC) diet for 24 weeks and from control mice fed regular Lean diet. Samples were embedded in optimal cutting temperature (OCT) compound and cryosectioned to preserve native composition. One section was screened under polarized light microscopy to identify birefringent crystals, whereas an adjacent section was analyzed by FT-IR imaging at the Canadian Light Source mid-infrared beamline. Hyperspectral images spanning 4000–900 cm^{-1} and high spatial resolution were collected for control and HFFC liver sections.

Results: Crystal-containing regions located inside lipid droplets display distinct absorption features characteristic of long, straight-chain carboxylic acids and carboxylate salts in the solid phase, suggesting the presence of solid amorphous or crystalline lipids or lipid esters. The crystals display spectral features of phospholipids and cholesterol esters. Spectral comparison with reference spectra of lipid standards, including free cholesterol, cholesteryl esters, phospholipids, and triglycerides will be used to evaluate the composition of these crystals. The crystalline spectral profiles were reproducible across multiple HFFC-fed mice liver samples. In contrast, livers from control diet–fed mice showed no birefringent crystals and lack the crystalline-associated spectral signature. Comparison with literature spectra suggest the crystal-bearing regions aligned more closely with cholesteryl esters and phospholipids than with free cholesterol or triglycerides. Collectively, these findings suggest cholesteryl esters may predominate in hepatic crystalline deposits under dietary cholesterol overload.

Conclusions: Infrared spectromicroscopy provides a powerful, label-free platform for characterizing cholesterol crystal composition in liver tissue. By integrating polarized light microscopy and FTIR analysis, we developed a workflow linking morphological detection with molecular definition. Preliminary results challenge the assumption that hepatic cholesterol crystals arise primarily from free cholesterol, instead implicating cholesteryl esters as a major component. This insight has implications for understanding lipid droplet biology, crystal stability, and the biochemical drivers of MASH. Ongoing studies will extend these observations through lipidomic validation and correlation with histological markers to further define how crystal composition may contribute to hepatic injury.

PhD Student

P-19

Loss of Hepatic MT1-MMP Alters Glucose-Lipid Metabolism and Reveals Sex-Specific Risks to Diet-Induced Metabolic Dysfunction

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Background: The liver coordinates glucose-lipid homeostasis through integrated nutrient and hormonal signaling. In type 2 diabetes (T2D), hepatic insulin signaling is impaired, accelerating hyperglycemia and dyslipidemia. Membrane type 1 matrix metalloproteinase (MT1-MMP/Mmp14) regulates receptor turnover, including the insulin receptor, and is implicated in diet-induced liver inflammation. Whether hepatocyte MT1-MMP orchestrates broader metabolic reprogramming and whether its effects differ by sex remains unknown.
Hypothesis: Based on our finding that hepatocyte Mmp14 loss in chow-fed mice elevates fasting glucose and lowers circulating insulin, we hypothesize that Mmp14 deficiency impairs peripheral insulin signaling and exacerbates glucose intolerance during diet-induced metabolic stress.
Methods and Results: Hepatocyte-specific Mmp14 knockout (Mmp14-HKO) and littermate control (Mmp14-Flox) mice were generated. Bulk RNA-seq was performed on livers from chow-fed animals to identify differentially expressed genes (DEGs; FDR<0.05) and enriched pathways. Mice were then fed a high-fat, high-cholesterol diet (HFHCD) for 8 weeks, with serial measurements of body weight and fasting blood glucose (FBG). Additionally, endpoint mice fat and lean mass compositions were measured using EchoMRI. Furthermore, endpoint mice livers were stained with hematoxylin and eosin (H&E) to investigate lipid accumulation within hepatocytes. Quantitative polymerase chain reaction (qPCR) was employed to measure altered transcript expression of key genes involved in lipogenesis and beta oxidation. Transcriptomic and physiological datasets were integrated to link molecular changes to systemic outcomes. In chow-fed Mmp14-HKO livers, bulk RNA-seq revealed transcriptional changes consistent with increased fatty acid handling and oxidation, alongside reduced insulin/IGF signaling, suggesting a shifting fasting metabolism away from glucose utilization and towards lipid handling. Under HFHC feeding, qPCR confirmed significantly decreased expression of the lipogenic enzyme fatty acid synthase (Fasn), while the β-oxidation marker carnitine palmitoyltransferase 1A (Cpt1a) trended toward upregulation (p=0.05). At the whole-body level, male Mmp14-HKO mice showed a 41.4% increase in body weight compared to controls (8.42 ± 1.14 g vs. 5.53 ± 1.18 g, p<0.05), with only modest changes in fasting blood glucose (FBG). EchoMRI analysis revealed increased fat mass and reduced lean mass in males, and both histological analysis of liver sections and direct measurements demonstrated significantly increased perigonadal fat mass with more lipid droplet accumulation within hepatocytes relative to controls (p<0.05). In contrast, female Mmp14-HKO mice gained weight comparable to controls but exhibited a 38.8% elevation in FBG compared to female controls (8.0 ± 0.8 vs. 5.4 ± 0.8 mmol/L, p<0.05). Together, these findings suggest that hepatocyte-specific Mmp14 loss reprograms fasting hepatic metabolism from glucose utilization towards lipid handling and results in divergent metabolic adaptations between sexes.
Conclusions: Hepatocyte-specific loss of Mmp14 reprograms liver metabolism toward increased fatty acid handling and reduced lipogenesis, yet this compensatory shift fails to prevent lipid accumulation under dietary stress. These changes drive sex-specific adaptations, with males developing greater adiposity and steatosis, while females exhibit impaired glucose regulation, underscoring MMP14 as a key regulator of hepatic metabolic balance.

Masters Student

P-20
Small Chemical Binders of GRP78 Reduce PCSK9 Secretion in Hepatocytes

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Background: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a circulating protein that increases the risk for hyperlipidemia and cardiovascular disease (CVD). Mechanistically, PCSK9 binds to low-density lipoprotein (LDL) cholesterol receptors on the liver surface, thereby inhibiting LDL cholesterol clearance, elevating cholesterol levels. PCSK9 expression is mainly regulated by sterol regulatory element binding protein 2 (SREBP2), an important transcriptional regulator of cholesterol synthesis located in the endoplasmic reticulum (ER). When cellular cholesterol levels are low SREBP2 is cleaved and transported to the nucleus activating downstream target genes, including PCSK9. Glucose regulated protein 78 (GRP78), a molecular chaperone, physically binds to SREBP2. We have recently reported that caffeine (CF) reduces serum PCSK9 by increasing ER Ca2+ levels, resulting in enhanced GRP78 binding affinity to SREBP2, retaining SREBP2 in the ER. As a result, SREBP2 activation is mitigated and PCSK9 expression is inhibited. Although CF shows a proposed mechanism to indirectly increase GRP78-SREBP2 complex stability our approach is to identify a direct method to target GRP78 independently of ER Ca2+ levels. Our drug development program has identified a library of novel small chemical binding molecules which bind to Leu98-Leu115 of GRP78 (AtomWise). These molecules are being screened to determine if they act directly on GRP78 to affect PCSK9 expression/secretion.
Hypothesis: We hypothesize that novel small chemical binders against GRP78 could potentially stabilize GRP78-SREBP2 binding directly, thereby retaining SREBP2 in the ER without affecting the levels of ER Ca2+. As a result, downstream gene targets of SREBP2 including PCSK9 would be reduced, allowing for increased LDL cholesterol clearance.
Methods: HuH7 cells were grown to 70% confluency prior to treatment with 10 μM GRP78 binding molecules in DMEM media. After a 12-hour time point Huh7 cell media was collected and an established PCSK9 ELISA Kit was used to measure secreted PCSK9 levels. An LDH assay was used to analyze cytotoxicity of small chemical GRP78 binders at varying concentrations ranging from 1 μM to 100 μM.
Results: After screening a library of 81 compounds we discovered four GRP78 binding molecules which showed a reduction of secreted PCSK9 levels ranging from 10-40% in HuH7 cells at a concentration of 10 μM. These binders did not show any signs of cytotoxicity at concentrations of up to 100 μM, indicating that the effect of PCSK9 levels was not due to cell injury . Well-established Fura 2-AM assays are now being utilized to determine whether our lead GRP78 binders increase ER Ca2+ levels which is required for mitigating PCSK9 expression and secretion by CF.
Conclusions: We have shown the ability of several GRP78 binders to reduce hepatocyte PCSK9 secretion. Currently we are working on analyzing the differential mRNA expression of PCSK9 and other SREBP2 target genes following treatment with our lead molecules. Ultimately, this shows a novel approach to treat hyperlipidemia and reduce PCSK9 levels by retaining SREBP2 in the ER.

Masters Student

P-21
Disruption of Lipid Metabolism Underlies Sepsis Pathology

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Background: Sepsis is a dysregulated immune response to infection that can lead to multi-organ failure. It is the leading cause of death for patients in intensive care units. Current treatment focuses on controlling the infection and hemodynamic stabilization. Therapies designed to mitigate the organ damage that arise in sepsis is a major unmet need. Emerging evidence suggests that lipid metabolism plays an important role in host defense during sepsis, offering potential therapeutic areas for sepsis treatment. Hence, it is crucial to elucidate the mechanism of lipid metabolism in the context of sepsis.
Hypothesis: Sepsis contributes to pathological outcomes and worsens survival outcomes by disrupting lipid metabolism.
Methods: To investigate the impact of sepsis on lipid metabolism, C57BL/6J mice were subjected to sepsis via intraperitoneal injection of 5 mg/kg lipopolysaccharide (LPS), 2.5×10^8 colony forming units (CFU) of live E. coli, or 6.5 mg of fecal slurry (a polymicrobial extract). Sixteen hours post-injection, liver tissue and blood were collected for lipid analysis. Plasma triglyceride concentrations were quantified using the Infinity Triglyceride reagent. Total cholesterol levels were measured using the Amplex Red Cholesterol Assay Kit. HDL-cholesterol was isolated by precipitating Apo-B-containing lipoproteins with 36% polyethylene glycol in 10 mM HEPES, followed by collection of the HDL-containing supernatant. Hepatic LDL receptor (LDLR) gene expression was assessed via qPCR, and protein levels were evaluated by Western blot. To measure hepatic VLDL secretion, mice were injected with either E. coli or fecal slurry. Sixteen hours later, mice received 1 g/kg of poloxamer 407, a lipoprotein lipase inhibitor. Blood samples were collected immediately before and 3 hours post poloxamer 407 injections. Plasma triglyceride levels then served as a surrogate marker for VLDL output. To assess the influence of a short-term high-fat diet and impaired lipid clearance on sepsis outcomes, LDLR-deficient mice were fed a high-fat, high-fructose, high-cholesterol (HFFC) diet for 7 days prior to LPS injection. Survival was monitored over a 72-hour period.
Results: Significant deviations in lipid profiles were observed. In septic mice, HDL-cholesterol levels decreased, while LDL- and VLDL-cholesterol levels increased. Hepatic VLDL secretion and LDLR gene and protein expression were also reduced. Plasma triglyceride and total cholesterol levels varied depending on the method used to induce sepsis. Wild-type mice fed a HFFC diet for 7 days prior to sepsis induction showed a marked increase in mortality. This effect was further exacerbated in LDLR-deficient mice on the same diet. Notably, LDLR deficiency alone did not affect sepsis survival.
Conclusions: Alterations in lipid parameters in septic mice indicate that sepsis disrupts lipid metabolism in the murine model. Also, short-term exposure to HFFC diet significantly worsens sepsis survival, with LDLR deficiency further exacerbating this effect. However, LDLR deficiency alone did not impact sepsis mortality, suggesting that the adverse outcome arises from a synergistic interaction between dietary factors and the absence of functional LDLR. Future investigations will focus on characterizing changes in lipoprotein composition and function during sepsis. Additional studies will explore the mechanisms by which short-term HFFC exposure increases sepsis mortality.

Masters Student

P-22

Serotonin, a downstream effector of GLP-2, enhances lacteal contractility and lymph flow

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Background: Glucagon-like peptide-2 (GLP-2) is known to exert some of its biological effects via the release of neurotransmitters, and in view of the absolute requirement for the enteric nervous system (ENS) demonstrated in our recent GLP-2-induced lipid mobilization studies, we aimed to identify the neurotransmitter that mediates GLP-2's effect on intestinal lipid mobilization. We also examined the role of VEGFR3 as an intermediate in the signaling cascade.
Hypothesis: Given the role of ENS, we hypothesized that GLP-2-indueced increases in lipid mobilization and lacteal contractility is regulated via secretion of serotonin.
Methods: Utilizing a rat lymph fistula model, 5 hours after an intraduodenal (i.d.) lipid bolus, the following intraperitoneal (i.p.) administrations were applied in two different sets of experiments: Experiment 1: 1) Placebo, 2) GLP-2, 3) GLP-2 + Ketanserin (serotonin receptor antagonist). Experiment 2: 1) Placebo, 2) Serotonin, 3) Serotonin + MAZ-51 (a VEGFR3 inhibitor), 4) Serotonin + SAR131675 (a second VEGFR3 inhibitor). Lymph flow and triglyceride (TG) output were assessed for 60 mins (Experiment 1) or 90 mins (Experiment 2) after administration. In another set of animals, GLP-2 or serotonin were administered i.p and blood samples were collected to quantify plasma serotonin concentration. Intravital imaging of a prospero-related homeobox 1-enhanced green fluorescent protein rat model was utilized to assess lacteal contractility after placebo or serotonin administration.
Results: We demonstrated that single-dose GLP-2 administration acutely increased serotonin concentration in plasma, serotonin enhanced lymph flow, lymph TG output and lacteal contractility, antagonism of the serotonin receptor decreases GLP-2-enhanced mesenteric lymph flow and TG output and inhibition of VEGFR3 abolished serotonin-induced lymph flow and TG output.
Conclusions: Our data suggests that the neurotransmitter serotonin mediates GLP-2's effect on intestinal lipid mobilization by enhancing lymph flow and lacteal contractility, and VEGFR3 is one of the downstream targets of serotonin involved in this cascade.

Postdoctoral Fellow

P-23

Elucidating the Physiological Function of Low-Density Lipoprotein Transcytosis in Arterial Endothelial Cells

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Background: In the initial stages of atherosclerosis, circulating LDL particles cross the arterial endothelial barrier and are deposited in the vessel intima. We and others have shown that this occurs by an intracellular traffic process known as transcytosis and is mediated by scavenger receptor B1 (SR-BI) and activin receptor-like kinase 1 (ALK1). Upon binding to LDL, SR-BI and ALK1 become internalized by membrane microdomains known as caveolae. Recent studies have confirmed that SR-BI and ALK-1-mediated transcytosis of LDL is essential for the development of atherosclerosis in murine models. However, whether LDL transcytosis serves any physiological functions remains unknown.

Hypothesis: SR-BI and ALK1 are structurally distinct transmembrane proteins with pleiotropic functions. The only known overlapping function between the two receptors is transcytosis of LDL. To elucidate the potential cellular function of LDL transcytosis, we analyzed the transcriptomic responses of primary human coronary artery endothelial cells depleted of each receptor. We reasoned that a common transcriptomic response to depletion of each receptor might suggest the function of LDL transcytosis.

Methods and Results: RNA sequencing of human coronary artery endothelial cells (HCAECs) depleted of SR-BI and ALK1 revealed several differentially expressed genes (DEGs) that are also identified in lipid droplet proteomes. This suggested that LDL transcytosis might be functionally related to lipid droplets (LDs). To confirm this, triglyceride (TAG)- or cholesteryl ester (CE)-rich LDs were induced in human coronary artery endothelial cells (HCAECs) with oleic acid or cholesterol respectively and transcytosis of LDL was measured. Cells with TAG-rich LDs performed significantly less LDL transcytosis compared to the control, while cells with CE-rich LDs exhibited a significant increase in LDL transcytosis. In contrast, depletion of LDL transcytosis receptors induced the formation of larger lipid droplets of both varieties. Fluorescent recovery after photobleaching analysis suggested TAG-rich LDs reduce caveolae mobility and thus limit uptake and transcytosis of LDL. Conversely, HCAECs with CE-rich LDs exhibited a significant decrease in LDL uptake yet increased exocytosis of LDL. This suggested that LDL internalized by transcytosis may remove cholesterol from lipid droplets. To prove this, HCAECs were loaded with deuterated cholesterol and confirmed to form CE-rich LDs by stimulated Raman scattering microscopy. Incubation of these cells with LDL caused a significant decrease in lipid droplet size over time. Collectively, these results support the notion that LDL undergoing transcytosis interacts functionally with lipid droplets. The detailed mechanisms are currently under investigation.

Conclusions: These results indicate a link between LDL transcytosis and regulation of intracellular storage of neutral lipids into LDs. Our ongoing studies aim to elucidate the underlying cellular mechanisms.

PhD Student

P-24

Mechanisms by which Glucocorticoids in the Nucleus Tractus Solitarius Stimulate Hepatic VLDL-TG Secretion

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Background: Obesity and type 2 diabetes (T2D) are escalating global health crises characterized by hyperlipidemia, including elevated plasma triglycerides (TG) and free fatty acids (FFAs), driven by insulin resistance and glucocorticoid (GC) dysregulation. While GCs are known to modulate lipid metabolism peripherally in white adipose tissue and the liver, their central actions, particularly in the brainstem nucleus tractus solitarius (NTS), remain less understood.
Hypothesis: We aimed to delineate pathways within the NTS which mediate the ability of NTS glucocorticoids to stimulate hepatic very low-density lipoprotein (VLDL)-TG secretion and investigate the requirement of sympathetic innervation for NTS GCs to stimulate hepatic VLDL-TG secretion.
Methods and Results: Male Sprague Dawley rats underwent stereotaxic cannulation to target the NTS and vascular catheterization to enable intravenous (i.v.) injections and arterial blood sampling in conscious, unrestrained rats. Animals were fasted for 10 hours to limit the presence of dietary lipids prior to VLDL-TG secretion experiments, in which i.v. Poloxamer 407 was administered with concurrent NTS infusions of: dexamethasone (DEX), a synthetic GC and glucocorticoid receptor (GR) agonist; BSA-conjugated DEX (BSAdex), a membrane-associated GC receptor agonist; 17-AAG (Hsp90i), a heat shock protein 90 inhibitor; Hsp90i+DEX; Hsp90i+BSAdex; bisindolylmaleimide I (BIMI), a protein kinase C (PKC) inhibitor; BIMI+DEX; or BIMI+BSAdex. Infusion of DEX or BSAdex into the NTS significantly increased VLDL-TG secretion rates compared to vehicle controls. Whereas NTS infusions of the inhibitors alone had no effect, NTS Hsp90i co-infusion blocked the increase in VLDL-TG secretion rate induced by NTS DEX but not BSAdex, and NTS BIMI co-infusion blocked the increase in VLDL-TG secretion rate induced by NTS BSAdex but not DEX. To assess the role of sympathetic innervation in mediating the hyperlipidemic effect of NTS GCs, a subset of rats receiving NTS DEX or NTS BSAdex also received an i.v. injection of an alpha- and beta-adrenergic receptor antagonist cocktail (SNSi), or 15% DMSO vehicle solution (control), prior to i.v. Poloxamer 407. NTS DEX or BSAdex infusion with i.v. DMSO elevated VLDL-TG and plasma FFAs, independent of plasma glucose changes. Notably, i.v. SNSi blocked both NTS DEX- and BSAdex-induced increases in VLDL, suggesting the requirement of sympathetic innervation in mediating the stimulatory effects of NTS GCs on VLDL-TG secretion. Whereas SNSi blocked the elevation in FFAs by NTS DEX, elevated FFAs were preserved in NTS BSAdex, suggesting that NTS GCs differentially regulate lipolysis. No significant changes in hepatic TG levels were observed. Both NTS DEX and BSAdex upregulated Fkbp5, Pdk4, and Dio2 gene expression in the NTS, but these were unaffected by SNSi.
Conclusions: We show that NTS DEX and BSAdex increase hepatic VLDL-TG secretion, but through distinct NTS signalling pathways: DEX acts via canonical GC receptors requiring HSP90, independent of PKC; while BSAdex signals through membrane GRs requiring PKC, independent of HSP90. SNSi negates the actions of both NTS DEX and BSAdex, highlighting the SNS as a critical mediator for NTS GC neurotransmission to the liver to stimulate VLDL-TG secretion. Elucidating these mechanisms offers insights into novel therapeutic targets for managing dyslipidemia in obesity and T2D.

Other Research Staff

P-25

The role of Prosaposin (Psap) in lipid metabolism and the development of atherosclerosis

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Background: Atherosclerotic plaques are rich in cholesterol, and elevated plasma LDL cholesterol (LDL-C) is a major risk factor. LDL-C is primarily cleared by LDL receptors (LDLR) in the liver via endocytosis. Prosaposin (PSAP), a precursor of 4 sphingolipid activator proteins, is essential for lysosomal glycosphingolipid degradation. It is proteolytically cleaved in the lysosomes to generate the smaller saposins A, B, C, and D, which act as detergents to facilitate glycosphingolipid degradation. Emerging evidence suggests PSAP may regulate LDLR degradation, though the mechanisms remain unclear. This study investigates how hepatocyte-specific PSAP deletion affects lipid metabolism and atherosclerosis progression, focusing on transcriptional regulation of lipid-related genes and the impact on LDLR protein abundance and plasma cholesterol levels.
Hypothesis: We hypothesized that PSAP regulates LDLR abundance in hepatocytes, thereby influencing plasma cholesterol levels and atherosclerosis progression.
Methods and Results: Hepatocyte-specific Psap knockout (Psap-HKO) mice were generated using the Cre-loxP system with albumin-Cre recombinase. Age-matched Psap flox (Psap-fl/fl) littermates served as controls. At 10 weeks, liver tissues were collected for lipid profiling, qRT-PCR, and immunoblotting. Hepatic expression of lipid regulatory genes, including Srebp2, Ldlr, Pcsk9, and Hmgcr, was analyzed. To assess atherosclerosis, Psap-HKO mice were crossed with mice with ApoE-null background and fed a Western diet for 6 weeks. Aortic lesion areas were quantified, and plasma total cholesterol and triglyceride levels were measured using enzymatic assays. Psap-HKO mice showed increased hepatic Ldlr and Srebp2 mRNA expression ($p < 0.05$), while Pcsk9 and Hmgcr remained unchanged. Despite elevated Ldlr transcripts, hepatic LDLR protein levels were reduced by 16% ($p = 0.1299$) in the male knockout group. In the atherosclerosis model, male Psap-HKO-ApoE-/- mice fed a Western diet for 6 weeks exhibited a 33% increase in plasma total cholesterol ($p = 0.1667$), 30% increase in plasma triglyceride ($p = 0.1627$) and a 47% larger aortic lesion area ($p = 0.3313$) versus controls. Also, LDLR protein levels were further decreased by 21% ($p = 0.1019$) in male Psap-HKO-ApoE-/- mice, suggesting a potential sex-specific regulatory effect of PSAP on LDLR protein stability.
Conclusions: Hepatic PSAP does not appear to play an anti-atherosclerotic role in ApoE-/- mice. However, further studies are required to confirm this finding.

PhD Student

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