Final Program

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Thursday Sept 26	4 pm	Check in
	5:00-6:30 pm	Dinner CLVS / CIHR/ICRH Meeting
	6:30-8:30 pm	**Session 1** ***Moderators: Bernardo Trigatti and Geoff Werstuck***
	6:30-6:45 pm	Opening Welcome
	6:45-7:30 pm	**Physician Scientist Lecture: Dr Warren Lee**, MD, PhD Introduced by Dr Bernardo Trigatti **LDL Transcytosis – Atherosclerosis by a Thousand Cuts?**
	7:30-8:15 pm	Oral presentations from abstracts
	7:30-7:45 pm	**O1*** **Alexander S. Qian**, Postdoctoral Fellow, Trigatti Lab, McMaster University Apolipoprotein A1 deficiency increases Macrophage Apoptosis and Necrotic Core Development in Atherosclerotic Plaques in a Bim dependent manner.
	7:45-8:00 pm	**O2*** **Julia M. St. John**, PhD Student, Boffa and Koschinsky Labs, Western University Understanding the complex role of elevated lipoprotein(a) in atherosclerosis.
	8:00-8:15 pm	**O3*** **Jae Hyun Byun**, MD/PhD student, Daskalopoulou Lab, Research Institute of McGill University Health Centre Increased Testosterone and Reduced Myofibroblast-like Smooth Muscle Cells are associated with Carotid Atherosclerotic Plaque Instability

Friday Sept 27	7:30-8:30 am	Breakfast
	8:30-10 am	**Session 2** ***Moderators: Colin Kretz and Gordon Francis***
	8:30-9:15 am	**Simone Pierre Noel Lecture: Dr Dawei Zhang**, PhD Introduced by Dr Gordon Francis **Regulatory mechanisms and therapeutic potential of low-density lipoprotein metabolism**
	9:15-10:00 am	Oral presentations from abstracts
	9:15-9:30 am	**O4** **Yosdel Soto,** Proctor Lab, University of Alberta Characterization of Physiochemical Properties and Efficacy of new variants of an P3R99 anti-atherosclerosis monoclonal antibody derived from CHO-K1 and HEK293 cell lines.
	9:30-9:45 am	**O5*** **Maria Elishaev**, PhD Student, Wang Lab, University of British Columbia Mapping cell phenotypes and microenvironment in human atherosclerotic lesions using multiplex imaging.
	9:45-10:00 am	**O6*** **Ann Kuganathan**, PhD Student, Krepinsky Lab, McMaster University Follistatin Improves Vascular Function by Regulating Perivascular Adipose Tissue in Essential Hypertension
	10-10:30 am	Break

Friday Sept 27	**10:30-12:00 pm**	**Session 3** ***Moderators: Marlys Koschinsky and Emily Day***
	10:30-11:15	**2024 CIHR-ICRH Distinguished Lecture/CSATVB Scientific Excellence Award in Blood and Blood Vessel Sciences:** **Dr Patricia Liaw**, PhD Introduced by Dr Ariane Marelli, CIHR-ICRH Director **Therapeutic strategies to target immunothrombosis in sepsis**
	11:15-12:00 pm	Oral presentations from abstracts
	11:15-11:30 am	**O7*** **Cameron Stotts**, PhD Student, Rayner Lab, University of Ottawa Heart Institute Pneumonia induces long-term changes in immune cells in atherosclerosis
	11:30-11:45 am	**O8*** **Jonathan O'Connor Miranda**, PhD Student, Lehoux Lab, McGill University Regulation of gut microbiota by D-Mannose is associated with reduced atherosclerosis in female ApoE-/- mice.
	11:45-12:00 pm	**O9*** **B. Sumayyah H. Sokeechand,** PhD Student, Trigatti Lab, McMaster University Investigating the role of Interleukin-15 receptor alpha in atherosclerosis
	12:00-1:00pm	Lunch

Friday Sept 27	**1:00-3:00pm**	**Session 4** ***Moderators: May Faraj and Tom Lagace***
	1:00-1:45 pm	**2024 CIHR-ICRH/CSATVB Mid-Career Excellence Award in Blood and Blood Research:** **Dr Jason Fish,** PhD Introduced by Dr Ariane Marelli, CIHR-ICRH Director **Oncogenic signaling in the endothelium drives arteriovenous malformations**
	1:45-2:30 pm	Oral presentations from abstracts
	1:45-2:00 pm	**O10*** **Andria Henry,** PhD Student, Lee Lab, University of Toronto. Lipoprotein(a) and the Arterial Endothelium: Elucidating Mechanisms of Residual Cardiovascular Risk
	2:00-2:15 pm	**O11*** **Mark De Leon**, Masters Student, Werstuck Lab, McMaster University. Investigating the Role of Angiogenesis on Atherosclerotic Pathogenesis in Diabetic Murine Models
	2:15-2:30 pm	**O12*** **Rex Huang**, Masters Student, Kretz Lab, McMaster University Degradation-resistant ADAMTS13 Variants exhibit improved stability and functional activity
	2:30-3:00 pm	**Jean Davignon Lecture Dr Nabil G. Seidah,** PhD Introduced by Dr May Faraj **The unique and complementary roles of PCSK9 and PCSK7 in cardiovascular diseases and cancer/metastasis**
	3:00-4:00 pm	CLC Business Meeting
	4-5:30 pm	**Poster Session 1** (judging of even numbered posters)
	6:00pm	CSATVB Executive Meeting (by invitation) Dinner on your own

Saturday Sept 28	7:30-8:30 am	Breakfast
	8:30-10 am	**Session 5** ***Moderators: Erin Mulvihill and Robin Duncan***
	8:30-9:15 am	**CLVS Stewart Whitman New Investigator Award Lecture: Dr Changting Xiao**, PhD Introduced by Dr Erin Mulvihill **Lipid Handling and Gut Hormones at the Crossroads of Gut**
	9:15-10:00 am	Oral presentations from abstracts
		O13* **Zarafshan Khan**, Masters Student, Liaw Lab, McMaster University Investigating the Optimal Time of Administration of Heparin that will Improve Survival in a Murine Model of Sepsis
		O14* **Rida Khan**, Masters Student, Devlin Lab, University of British Columbia Investigating molecular changes to unravel the chronology of cardiovascular damage in type 1 diabetes.
		O15* **Kalsha H. Diaguarachchige De Silva**, PhD Student, Duncan Lab, University of Waterloo The effects of chronic psilocybin administration in high fat diet-fed male and female C57BL/6J mice
	10-10:30	Break

FINAL PROGRAM

Saturday Sept 28	**10:30-12:30 pm**	**Session 6** ***Moderators: Nica Borradaile and Morgan Fullerton***
	10:30-11:30 am	**Rubenstein Lecture – Dr Ana Maria Cuervo**, MD, PhD Introduced by Dr Nica Borradaile **Chaperone-mediated autophagy: regulating metabolic adaptation one protein at a time**
	11:30-12:15 am	Oral presentations from abstracts
		O16* **Tamana Yousof,** Postdoctoral Fellow, Austin Lab, McMaster University Loss of TDAG51 attenuates methionine and choline deficient diet-induced ER stress and liver injury
		O17* **Ali A. Abdalbari,** Masters Student, Mulvihill Lab University of Ottawa Heart Institute Unveiling the interplay of DPP4 and EPDR1 in obesity: Implications for inflammation and atherosclerosis
		O18* **Peter Amadi,** Postdoctoral Fellow, Zhang Lab, University of Alberta The role of membrane type 1- matrix metalloproteinase in the development of liver fibrosis
	12:30-3:30 pm	**CIHR-ICRH/CSATVB** **Early Career Forum (includes lunch)**
	3:30-4 pm	CSATVB Annual General Meeting
	4-5:30 pm	**Poster Session 2** (judging of odd numbered posters)
	6pm	**CLVS Dinner**
Sunday Sept 29	**Departure**	Breakfast on your own

Keynote Lectures

Physician Scientist Lecture

Dr Warren Lee, University of Toronto

LDL Transcytosis – Atherosclerosis by a Thousand Cuts?

Simon Pierre Noel Lecture

Dr Dawei Zhang, University of Alberta

Regulatory mechanisms and therapeutic potential of low-density lipoprotein metabolism

2024 CIHR-ICRH Distinguished Lecture/CSATVB Scientific Excellence Award in Blood and Blood Vessel Sciences:

Dr Patricia Liaw, McMaster University.

Therapeutic strategies to target immunothrombosis in sepsis

2024 CIHR-ICRH/CSATVB Mid-Career Excellence Award in Blood and Blood Research

Dr Jason Fish, University of Toronto

Oncogenic signaling in the endothelium drives arteriovenous malformations

Jean Davignon Lecture

Dr Nabil G Seidah, Institut de Recherches Cliniques de Montréal and Université de Montréal

The unique and complementary roles of PCSK9 and PCSK7 in cardiovascular diseases and cancer/metastasis

CLVS Stewart Whitman New Investigator Award

Dr Changting Xiao, University of Saskatchewan.

Lipid Handling and Gut Hormones at the Crossroads of Gut

Rubenstein Lecture

Dr. Ana Maria Cuervo, Albert Einstein College of Medicine

Chaperone-mediated autophagy: regulating metabolic adaptation one protein at a time

Oral Presentations from Submitted Abstracts

O1 Alexander S. Qian, Postdoctoral Fellow, Bernardo Trigatti Lab, McMaster University
APOLIPOPROTEIN A1 DEFICIENCY INCREASES MACROPHAGE APOPTOSIS AND NECROTIC CORE DEVELOPMENT IN ATHEROSCLEROTIC PLAQUES IN A BIM DEPENDENT MANNER.

O2 Julia M. St. John, PhD Student. Michael B. Boffa and Marlys L. Koschinsky Lab, Western University
UNDERSTANDING THE COMPLEX ROLE OF ELEVATED LIPOPROTEIN(A) IN ATHEROSCLEROSIS.

O3 Jae Hyun Byun, MD/PhD student, Styliani S. Daskalopoulou Lab, Research Institute of McGill University Health Centre
INCREASED TESTOSTERONE AND REDUCED MYOFIBROBLAST-LIKE SMOOTH MUSCLE CELLS ARE ASSOCIATED WITH CAROTID ATHEROSCLEROTIC PLAQUE INSTABILITY

O4 Yosdel Soto, Spencer Proctor Lab University of Alberta
CHARACTERIZATION OF PHYSIOCHEMICAL PROPERTIES AND EFFICACY OF NEW VARIANTS OF AN P3R99 ANTI-ATHEROSCLEROSIS MONOCLONAL ANTIBODY DERIVED FROM CHO-K1 AND HEK293 CELL LINES.

O5 Maria Elishaev, PhD Student, Ying Wang Lab, University of British Columbia
MAPPING CELL PHENOTYPES AND MICROENVIRONMENT IN HUMAN ATHEROSCLEROTIC LESIONS USING MULTIPLEX IMAGING.

O6 Ann Kuganathan, PhD Student, Joan Krepinsky Lab, McMaster University
FOLLISTATIN IMPROVES VASCULAR FUNCTION BY REGULATING PERIVASCULAR ADIPOSE TISSUE IN ESSENTIAL HYPERTENSION

O7 Cameron Stotts, PhD Student, Katey Rayner Lab, University of Ottawa Heart Institute
PNEUMONIA INDUCES LONG-TERM CHANGES IN IMMUNE CELLS IN ATHEROSCLEROSIS

O8 Jonathan O'Connor Miranda, PhD Student, Stephanie Lehoux Lab, McGill University
REGULATION OF GUT MICROBIOTA BY D-MANNOSE IS ASSOCIATED WITH REDUCED ATHEROSCLEROSIS IN FEMALE APOE-/- MICE.

O9 B. Sumayyah. H Sokeechand, PhD Student, Bernardo Trigatti Lab, McMaster University
INVESTIGATING THE ROLE OF INTERLEUKIN-15 RECEPTOR ALPHA IN ATHEROSCLEROSIS

Oral Presentations from Submitted Abstracts

O10 Andria Henry, PhD Student, Warren L. Lee Lab, University of Toronto.
LIPOPROTEIN(a) AND THE ARTERIAL ENDOTHELIUM: ELUCIDATING MECHANISMS OF RESIDUAL CARDIOVASCULAR RISK

O11 Mark De Leon, Masters Student, Geoff Werstuck Lab, McMaster University.
INVESTIGATING THE ROLE OF ANGIOGENESIS ON ATHEROSCLEROTIC PATHOGENESIS IN DIABETIC MURINE MODELS

O12 Rex Huang, Masters Student, Colin Kretz Lab, McMaster University
DEGRADATION-RESISTANT ADAMTS13 VARIANTS EXHIBIT IMPROVED STABILITY AND FUNCTIONAL ACTIVITY

O13 Zarafshan Khan, Masters Student, Patricia Liaw lab, McMaster University
INVESTIGATING THE OPTIMAL TIME OF ADMINISTRATION OF HEPARIN THAT WILL IMPROVE SURVIVAL IN A MURINE MODEL OF SEPSIS

O14 Rida Khan, Masters Student, Angela M. Devlin Lab, University of British Columbia
INVESTIGATING MOLECULAR CHANGES TO UNRAVEL THE CHRONOLOGY OF CARDIOVASCULAR DAMAGE IN TYPE 1 DIABETES.

O15 Kalsha H. Diaguarachchige De Silva, PhD Student, Robin E. Duncan Lab, University of Waterloo
THE EFFECTS OF CHRONIC PSILOCYBIN ADMINISTRATION IN HIGH FAT DIET-FED MALE AND FEMALE C57BL/6J MICE

O16 Tamana Yousof, Postdoctoral Fellow, Richard Austin Lab McMaster University
LOSS OF TDAG51 ATTENUATES METHIONINE AND CHOLINE DEFICIENT DIET-INDUCED ER STRESS AND LIVER INJURY

O17 Ali A. Abdalbari, Masters Student, Erin E. Mulvihill Lab University of Ottawa Heart Institute
UNVEILING THE INTERPLAY OF DPP4 AND EPDR1 IN OBESITY: IMPLICATIONS FOR INFLAMMATION AND ATHEROSCLEROSIS

O18 Peter Amadi, Postdoctoral Fellow, Dawei Zhang Lab, University of Alberta
THE ROLE OF MEMBRANE TYPE 1- MATRIX METALLOPROTEINASE IN THE DEVELOPMENT OF LIVER FIBROSIS

Poster Presentations

All posters to be displayed for both poster sessions

Even numbered posters: Presenters to be at posters during Friday poster session

Odd numbered posters: Presenters to be at posters during Saturday poster session.

P1** **Xiaoying Wu**, Jesse Batara, Mahua Ghosh, Paolo Raggi, Harald Becher, Donna Vine. ACCELERATED PROGRESSION OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN YOUNG WOMEN WITH POLYCYSTIC OVARY SYNDROME

P2 **Aishah AL-Jarallah**, Samah Kalakh, Saghir Akhtar, Mariam HM Yousif. HDL ATTENUATES ANG II-AT1R-EGFR SIGNALING AND REVERSES VASCULAR REMODELING IN SPONTANEOUSLY HYPERTENSIVE RATS

P3 Ashkan Hashemi1, Ming Rong Liu1, John Z. Chan1, Michelle V. Tomczewski1, Alex D. Cocco1, Douglas Strathdee2, Ken Stark1, and **Robin E. Duncan1** CHARACTERIZATION OF PLAAT1-/- MICE

P4 Enoch Yu (1), Matthew Holding (2), Rex Huang (3), Andrew Chan(1), Cherie Teney (1), **Colin A. Kretz**(1,3) DEEP PROTEASE PROFILING DECONVOLUTES PROTEASE SIGNATURES RELEASED FROM ACTIVATED NEUTROPHILS

P5* **Justine Fricher**, Rianne Mahiout, Simon Bissonnette, Valérie Lamantia, May FARAJ EPA AND DHA ATTENUATE, BUT DOES NOT ELIMINATE, THE ASSOCIATION OF PLASMA APOB-TO-PCSK9 RATIO TO METABOLIC RISK IN HUMANS

P6 Conor O'Dwyer, Tyler K.T. Smith, Peyman Ghorbani, Jianfan Nie, Madison Girouard and **Morgan D. Fullerton*** ENERGY AND NUTRIENT SENSING PATHWAYS AS REGULATORS OF METABOLIC DISEASE

P7** **Justin Clark**, Frances Sutherland, Julia St. John, Michael Boffa, Marlys Koschinsky. LIPOPROTEIN(A) PROMOTES THROMBOSIS THROUGH EFFECTS ON PLATELET AGGREGATION

P8** **Inbar A. Habaz**, Ting Xiong, Bernardo L. Trigatti. INVOLVEMENT OF FIBROBLAST ACTIVATION PROTEIN IN MYOCARDIAL REMODELLING DURING INFARCTION IN A MOUSE MODEL OF CORONARY ARTERY DISEASE

P9 **Talin Ebrahimian**, Jonathan O'Connor Miranda, France Dierick, Maria Kotsiopriftis, Jaclyn Itzcovitch, Anthony Parent, Julie Boddaert, Stephanie Lehoux MANNOSE ORAL SUPPLEMENTATION DECREASES ATHEROSCLEROSIS, INFLAMMATORY IMMUNE CELL RECRUITMENT AND IMPROVES MITOCHONDRIAL FUNCTION IN APOE-/- MICE

P10* **Julien Wourms**, Mantash Grewal, Boyan Vasilev, Bryan M. Lum, Jessica T.Y. Yue. GLUCAGON SIGNALLING IN THE NUCLEUS OF THE SOLITARY IN THE REGULATION OF HEPATIC TRIGLYCERIDE SECRETION IN A MODEL OF TYPE 2 DIABETES

P11*** **Majid Mufaqam Syed-Abdul***, Lili Tian*, Gary F. Lewis. ACTIVATION OF VEGFR3 AND MLC2 ARE CRITICAL FOR GLP-2 ENHANCEMENT OF CHYLOMICRON TRANSPORT

P12 Christopher Yuen, Zoe White, Angela M. Devlin, **Pascal Bernatchez**. EARLY ENDOTHELIAL FUNCTION PREVENTS CHRONIC DAMAGE IN DIABETES: UNRAVELLING PROTECTIVE ENDOTHELIAL-SPECIFIC MECHANISMS

Poster Presentations

All posters to be displayed for both poster sessions

Even numbered posters: Presenters to be at posters during Friday poster session

Odd numbered posters: Presenters to be at posters during Saturday poster session.

P13* **Wan Hei CHENG**, Samuel LEUNG, Yuancheng MAO, Maria ELISHAEV, Tiffany CHANG, Chi LAI, Amrit SINGH, Ying WANG. CHARACTERIZING INFLAMMATION IN CORONARY ARTERY DISEASE

P14** **Jessica Twumasi Ankrah**, Cyriel Huijer, Rachel Wilson, Xindi Zhao, Rosemary Yu, Nica Borradaile. SHARED REGULATION OF CELLULAR METABOLISM BY PROTEIN TRANSLATION ELONGATION FACTOR 1A (EEF1A) PARALOGS

P15. **Vittoria Baht**, Katrina Besler, Teddy Chan, Sima Allahverdian, Gordon. A. Francis. Mobilization of Cholesteryl Esters in Smooth Muscle Cell Foam Cells by Overexpression of Lysosomal Acid Lipase

P16*** **Derek W. Stouth**, Matthew Sguazzin, Guillaume Paré, Mark A. Tarnopolsky, Jakob Magolan, Richard C. Austin. CAFFEINE-INSPIRED PCSK9 INHIBITORS FOR THE TREATMENT OF CARDIOVASCULAR DISEASES

P17** **Govind Gill**, Peter Amadi, Suha Jarad, Hongmei Gu, Aliun Gaowa, Moattar Latif, Dawei Zhang. MONOOXYGENASE X REGULATES COLLAGEN ACCUMULATION IN RESPONSE TO CHEMICAL-INJURY INDUCED LIVER FIBROSIS.

P18**. **Suha Jarad**, Hongmei Gu, Govind Gill, Peter Amadi, Da-wei Zhang. VASCULAR SMOOTH MUSCLE CELL SPECIFIC MT1-MMP CAN DECREASE ATHEROSCLEROSIS PROGRESSION IN LDLR KO MICE

P19* George E.G. Kluck, **Temitope R. Lekuti**, Bernardo L. Trigatti. PDZK1 DEFICIENCY INCREASES DIET INDUCED CORONARY ARTERY ATHEROSCLEROSIS, MYOCARDIAL FIBROSIS AND LEFT VENTRICULAR DYSFUNCTION IN LDLRKO/KO MICE.

P20** **Peter A. Andrisani**, Cherie Teney, Colin A. Kretz. INVESTIGATING MECHANISMS OF ADAMTS13 METALLOPROTEASE DOMAIN LATENCY

P21*** **Devika Jayawardena**, Zachary Easton, Daniel Hardy, Michael Boffa, Marlys L. Koschinsky. LP(A) INDUCES A PRO-GLYCOLYTIC AND PRO-INFLAMMATORY RESPONSE IN CORONARY ARTERY AND AORTIC VALVE ENDOTHELIAL CELLS, WITH ALTERED METABOLISM AND REACTIVE OXYGEN SPECIES GENERATION.

P22* **Simon J. Ofiara**, Bryan Heit, Michael B. Boffa, Marlys L. Koschinsky. INTRACELLULAR PLAYERS INVOLVED IN LP(A) BIOSYNTHESIS AND SECRETORY TRAFFICKING

P23. Zoe White, **Pascal Bernatchez**. ARE ANEURYSMS AND OTHER AORTOPATHIES DISEASES OF THE ENDOTHELIUM? INTRODUCING THE NITRIC OXIDE (NO)-DEPENDENT THERAPEUTIC ENDOTHELIAL FUNCTION RESERVE.

P24** **Jeong-Ah Yoo**, Bernardo Trigatti. THE ADMINISTRATION OF APOA1 SELECTIVELY PROTECTS CARDIOMYOCYTES WHILE MAINTAINING THE CHEMOTHERAPEUTIC EFFICACY OF DOX IN THE SAME PRE-CLINICAL MODEL.

Abstracts of Keynote Lectures

Dr Warren Lee Physician Scientist Lecture

ICU Physician and Tier two Canada Research Chair in Mechanisms of Endothelial Permeability, University of Toronto

LDL Transcytosis – Atherosclerosis by a Thousand Cuts?

The accumulation of low density lipoprotein (LDL) in the intimal layer of arteries represents a first and pivotal step in atherogenesis. Every artery in the body is lined with endothelial cells and the accumulation of LDL in the intima indicates that circulating LDL has crossed the endothelial barrier. Recent studies have highlighted that LDL passes through individual cells by a form of vesicular traffic known as **transcytosis**. However, because of long-standing technical hurdles, remarkably little is known about this fundamental step in atherogenesis.

In pioneering work Lee's lab developed a novel live cell imaging assay that specifically measures endothelial transcytosis and identified 2 receptors, SR-BI and ALK1, that perform transcytosis of LDL. They showed that human coronary artery endothelial cells from premenopausal females perform less LDL transcytosis than cells from males, and that physiological concentrations of estrogen significantly attenuate LDL transcytosis by the cells from males. These data established that LDL transcytosis is a regulated process and are consistent with the observation that premenopausal women have a lower risk of coronary artery disease than age-matched men. Recent studies by Lee and others have established that LDL transcytosis by SR-BI and ALK1 is induced by inflammation and required for atherogenesis both early and late in the disease, catalyzing interest in this previously neglected field.

Despite these recent advances, almost nothing is known about what occurs within the cell after LDL is internalized. Lee's group is working to characterize the specific intracellular route taken by LDL during transcytosis across human coronary artery endothelial cells. Determining how LDL transcytosis is regulated is now the subject of intensive investigation with the goal of identifying novel therapeutic targets.

Dr Dawei Zhang—Simon Pierre Noel Lecture

Professor, Department of Pediatrics at the University of Alberta

Regulatory mechanisms and therapeutic potential of low-density lipoprotein metabolism

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide. Plasma levels of cholesterol, especially low-density lipoprotein cholesterol (LDL-C), are positively correlated to CVD risk. Lowering plasma LDL-C significantly reduces CVD risk. LDL is produced through catabolism of very low-density lipoprotein (VLDL), a triglyceride-rich lipoprotein that is produced primarily by the liver. Triglycerides on circulating VLDL are hydrolyzed by lipoprotein lipase to form intermediate-density lipoprotein, which is then converted to LDL. In humans, LDL can accept cholesteryl esters from high-density lipoprotein (HDL) via cholesteryl ester transfer protein (CETP).

LDL is cleared from circulation primarily through the hepatic LDL receptor (LDLR). Mutations in LDLR cause familial hypercholesterolemia, which is characterized by elevated plasma levels of LDL cholesterol and increased CVD risk. For example, statins, the most-prescribed drug to reduce plasma lipid levels, increase LDLR expression and subsequently enhance LDL-C clearance.

I will discuss the role of the cargo receptor Surfeit 4 (Surf4) in lipid metabolism, focusing on how Surf4 mediates lipoprotein secretion. I will also discuss the molecular mechanisms regulating LDLR expression. Understanding these mechanisms may pave the way for the

development of new lipid-lowering therapies for patients with hyperlipidemia who are intolerant to existing therapies or unable to effectively manage their condition with existing therapies.

Dr Patricia Liaw, PhD. **2024 CIHR-ICRH Distinguished Lecture/CSATVB Scientific Excellence Award in Blood and Blood Vessel Sciences:**

Professor and Jack Hirsh & Clive Kearon Chair in Thrombosis Research in the Department of Medicine, Division of Hematology & Thromboembolism at McMaster University.

Therapeutic strategies to target immunothrombosis in sepsis

Sepsis is a life-threatening complication of infection in which a dysregulated host response precipitates multi-organ dysfunction. Immunothrombosis is considered a beneficial mechanism of innate immunity in which immune cells actively participate in the formation of blood clots. Immunothrombosis generates an intravascular scaffold that facilitates the containment and destruction of pathogens, However, if uncontrolled, immunothrombosis is a driver of microvascular thrombosis which causes tissue hypoperfusion and organ failure. Despite decades of research, there is no effective anti-sepsis therapy and the mortality rate for sepsis patients remains high (~20%). My presentation will focus on novel therapeutic strategies to target immunothrombosis in sepsis. I will also describe approaches to improve the success of clinical trials in sepsis, with focus on the optimal timing and dose of therapies as well as precision medicine.

Dr Jason Fish, PhD. 2024 CIHR-ICRH/CSATVB Mid-Career Excellence Award in Blood and Blood Research

Senior Scientist, Toronto General Research Institute and Professor, Department of Pathobiology and Laboratory Medicine, University of Toronto

Oncogenic signaling in the endothelium drives arteriovenous malformations

Brain arteriovenous malformations are abnormal direct connections between arteries and veins that are prone to rupture and are the leading cause of stroke in young people. The genetic cause of these sporadic lesions was unknown until our collaborative discovery in 2018 that somatic activating mutations in the KRAS gene are found in the endothelium of lesions in the majority of patients. We have developed cellular, zebrafish and mouse models to understand how oncogenic signaling by KRAS can lead to brain arteriovenous malformations. We have also used our models to develop new therapies for this disease.

Dr Nabil G Seidah, Jean Davignon Lecture

Professor and Director of the Biochemical Neuroendocrinology Research Unit in the Centre de santé cardiométabolique at the Institut de Recherches Cliniques de Montreal and Professor at the Université de Montréal

The unique and complementary roles of PCSK9 and PCSK7 in cardiovascular diseases and cancer/metastasis

BACKGROUND: PCSK9, the 9th member of the proprotein convertases (PCSK) family, was discovered in 2003 and has since merged as central PCSK implicated in the non-enzymatic regulation of LDLc, due to its ability to enhance the degradation of the LDLR. PCSK9 is now implicated in various pathologies including atherosclerosis, inflammation, myocardial infarction, and cancer. Clinically approved inhibitors/silencers of PCSK9 include mAb, siRNA,

small molecules, and CRISPR/Cas. PCSK7, the 7th member of the PCSK family, was discovered in 1996, but its physiological functions remained obscure for a long time. Only recently have epidemiological and PCSK7 silencing strategies using KO and ASOs uncovered a non-enzymatic in vivo role of PCSK7 in triglyceride metabolism and regulation of apoB secretion, suggesting that silencing PCSK7 is a potential new treatment for non-alcoholic Fatty Liver Disease (NAFLD, MAFLD).

HYPOTHESIS: In view of the sequence and structural homology of PCSK9 and PCSK7, as well as their in vivo implication in lipid metabolism and cancer, we hypothesized that the combination of inhibitors/silencers of PCSK9 and PCSK7 may synergize the treatments of various pathologies including cardiovascular diseases and cancer/metastasis.

METHODS AND RESULTS: We generated human/mouse hepatic cell lines lacking one or both convertases as well as viable and healthy Pcsk9-/-, Pcsk7-/- and double KO mice. We discovered that PCSK7 binds apoB100 in the endoplasmic reticulum (ER) and enhances its secretion (chaperone/escort effect). On the other hand, the loss of PCSK7 leads to apoB degradation in the ER, triggering an unfolded protein response, autophagy, and β-oxidation, eventually reducing hepatic lipid accumulation. We then proved that Pcsk7-/- mice or mice treated with antisense-oligonucleotides (ASOs) specifically targeted to hepatocytes (N-acetylgalactosamine GalNac-modification) exhibited a safe and significantly faster recovery from NAFLD compared to wild type or non-treated mice. Next, we turned our attention to the highly invasive and deadly colorectal and pancreatic cancers and showed that in mice silencing either convertase alone significantly reduced tumor growth and/or metastasis to liver. This protection was highly enhanced in double KO mice where cancer growth and liver metastasis were virtually eliminated. The common protective mechanism observed upon silencing one or both genes seems to converge towards a more effective activation of CD8+ T cells and their infiltration of tumors.

CONCLUSIONS: In mice and humans inhibition of either PCSK7 or PCSK9 nonenzymatically reduces hepatic TG accumulation and safely enhance the liver recovery from NAFLD, suggesting that it is a safe and effective therapy for liver steatosis. Inhibition/silencing of either PCSK9, PCSK7, or both significantly reduced tumor growth and the extent of their metastasis to liver. This suggests that a combination of inhibitors of PCSK9 and PCSK9 may represent safe and powerful strategy to be combined with state of the art treatments of proliferative pathologies.

Dr Changting Xiao: Recipient of the CLVS Stewart Whitman New Investigator Award

Assistant Professor, Department of Anatomy, Physiology, and Pharmacology at the College of Medicine, University of Saskatchewan.

Lipid Handling and Gut Hormones at the Crossroads of Gut

Cardiovascular diseases are the leading cause of death in patients with chronic metabolic diseases (e.g. type 2 diabetes, obesity). These conditions are commonly associated with atherosclerotic dyslipidemia, at the center of which is abnormal metabolism of triglyceride-rich lipoproteins. The gut is a vital organ for dietary fat digestion and absorption. Mishandling of lipids and dysregulated lipoprotein metabolism in the gut contributes to atherosclerotic dyslipidemia. Regulation of gut lipid handling is complex. Previous research showed that lipoprotein synthesis and secretion in the gut is regulated by nutrients, nutraceuticals and hormones, including the gut hormones glucagon-like peptide-1 and -2. Recent clinical trials demonstrate significant benefits of GLP-1-based anti-diabetes and anti-obesity therapies on cardiovascular outcomes. One of our research interests is to elucidate the mechanisms whereby gut hormones regulate lipid handling in the gut. This presentation aims to provide an update on the development in this area. Better understanding gut lipid handling and the mechanisms of

action of gut hormones may help develop novel therapeutic approaches towards dyslipidemia and atherosclerotic cardiovascular diseases.

Dr. Ana Maria Cuervo, MD PhD Rubenstein Lecturer

Distinguished Professor, and Robert and Reneé Belfer Chair for the Study of Neurodegenerative Diseases, Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY

Chaperone-mediated autophagy: regulating metabolic adaptation one protein at a time

Selective autophagy contributes to maintenance of the cellular energetic balance as well as to protein and organelle quality control. We have been investigating the role of chaperone-mediated autophagy (CMA), a form of selective degradation of proteins in lysosomes, in the maintenance of energy homeostasis and in the cellular response to metabolic stressors. One previously ignored function of this type of autophagy is its ability to selectively degraded fully functional proteins to terminate their function, and consequently contribute to modulate the cellular processes that those proteins are involved. Control of glucose and lipid metabolism and of the different mechanisms that contribute to the cellular and organism energetic balance is proven to be tightly linked to CMA.

We have found that CMA activation is part of the first front of defense against the metabolic derangements associated to nutritional challenges and to aging. In this talk, I will provide examples of how CMA regulates metabolic switches through selective and timely degradation of proteins implicated in glucose and lipid metabolism. Through similar selective degradation, we have identified a mechanism by which autophagy also modulates mitochondrial enzymatic load without changing the overall cellular mitochondrial content. Lastly, I will also comment on the protective function of CMA against metabolic stress.

Considering the well-reported decline in CMA activity with age and during dietary challenges, we propose that modulation of this type of selective autophagy may be an effective intervention in common metabolic disorders. I will provide examples of our current efforts to genetically and chemically restore CMA in these conditions.

Abstracts of Short Oral Presentations

O1 APOLIPOPROTEIN A1 DEFICIENCY INCREASES MACROPHAGE APOPTOSIS AND NECROTIC CORE DEVELOPMENT IN ATHEROSCLEROTIC PLAQUES IN A BIM DEPENDENT MANNER.

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McMaster University

BACKGROUND: In advanced atherosclerotic lesions, macrophage apoptosis contributes to plaque progression and the formation of necrotic cores, rendering plaques vulnerable to rupture. The pro-apoptotic protein Bim plays a crucial role in mediating apoptosis in macrophages under prolonged endoplasmic reticulum (ER) stress. High-density lipoprotein (HDL) has been shown to suppress macrophage apoptosis induced by ER stressors.

HYPOTHESIS: We hypothesize that apolipoprotein A1 (ApoA1) deficiency increases necrotic core growth and plaque apoptosis associated with an increase in pro-apoptotic protein Bim. Additionally, we hypothesize that deletion of bone marrow derived Bim reduces necrotic core growth and plaque apoptosis in atherosclerotic mice.

METHOD AND RESULTS: To investigate the impact of ApoA1 deficiency, associated with reduced HDL levels, on necrotic core growth and plaque apoptosis, we introduced ApoA1 deficiency into low-density lipoprotein receptor (LDLR) knockout mice and fed them a high-fat diet for 10 weeks. ApoA1-deficient LDLR knockout mice developed advanced plaques characterized by large necrotic cores, increased apoptosis, and elevated Bim expression in macrophages within the plaques. To assess whether deletion of Bim could mitigate this development, mice underwent bone marrow transplantation with bone marrow from either Bim-deficient mice or from mice with a deletion of myeloid-derived Bim driven by LyzM-cre. Inhibiting Bim in all bone marrow-derived cells led to leukocytosis, reductions in plasma cholesterol and triglyceride levels, and decreased plaque apoptosis, necrotic core, and plaque sizes in ApoA1 and LDLR double-knockout mice but not in LDLR knockout mice. Likewise, conditional deletion of Bim in the myeloid compartment of ApoA1 and LDLR double-knockout mice also reduced apoptosis, necrotic core sizes, and plaque sizes, without inducing leukocytosis or lowering plasma cholesterol levels.

CONCLUSIONS: These findings suggest that ApoA1 deficiency triggers apoptosis in myeloid cells through a Bim-dependent pathway, significantly contributing to the development of necrotic cores and the progression of atherosclerotic plaques.

O2 UNDERSTANDING THE COMPLEX ROLE OF ELEVATED LIPOPROTEIN(A) IN ATHEROSCLEROSIS

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Introduction: Elevated plasma levels of lipoprotein(a) (Lp(a)) (>125 nmol/L), are a causal and independent risk factor for atherosclerotic cardiovascular disease (ASCVD). However, the precise contribution of Lp(a) to ASCVD progression is poorly understood.

Methods: Using transgenic human Lp(a) mice (Tg(LPA+/0;APOB+/0)), that express genes for human apolipoprotein(a) (LPA) and human apoB100 (APOB), we assessed the impact of

pathogenic levels of Lp(a) (mean, 331.2 nmol/L males and 193.5 nmol/L females) in the setting of advanced atherosclerosis. Male and female Tg(LPA+/0;APOB+/0) mice and Tg(APOB+/0) controls (n=9-14/group) were injected once with AAV-CRISPR targeting the mouse LDL receptor gene and fed a high-fat, high-cholesterol diet for 24 weeks. The aortic sinus was collected and subjected to histopathological analysis. Circulating peripheral blood mononuclear cells were isolated from whole blood and flow cytometry was performed to characterize circulating immune cells (B-lymphocytes, neutrophils, T-lymphocytes and monocytes).

Results: Total plaque abundance, assessed by Oil-Red-O staining, was increased (19%, p=0.0317 and 25%, p=0.0637) in male and female Tg(LPA+/0;APOB+/0) mice respectively, compared to Tg(APOB+/0) mice. Calcium deposition was increased 3-fold in lesions from male Tg(LPA+/0;APOB+/0) mice (p=0.04061) with a significant increase in necrotic core area (p=0.0482) and collagen abundance (p=0.0409), when compared to Tg(APOB+/0) controls. No significant differences were observed in calcium staining or collagen abundance in female mice to date (only n=7-9/group); analysis of additional female mice from this study is ongoing. Flow cytometry of circulating immune cells from male Tg(LPA+/0;APOB+/0) mice demonstrated a shift toward a higher proportion of pro-inflammatory CD8a+ T-lymphocytes and a decrease in the proportion of anti-inflammatory Ly6Clo monocytes when compared to male Tg(APOB+/0) mice.

Conclusion: In the context of severe atherosclerosis, elevated levels of Lp(a) promote inflammation and the progression of atherosclerosis. Ongoing single cell sequencing of the atherosclerotic plaques from this study will provide further insight into the role of Lp(a) in ASCVD.

O3 INCREASED TESTOSTERONE AND REDUCED MYOFIBROBLAST-LIKE SMOOTH MUSCLE CELLS ARE ASSOCIATED WITH CAROTID ATHEROSCLEROTIC PLAQUE INSTABILITY

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Background: Cardiovascular disease remains the leading cause of death worldwide. Unstable atherosclerotic plaques that reside in the carotid arteries are major etiological factors known to increase the incidence of ischemic strokes. Moreover, sex differences exist in plaque morphology and composition; males are more prone to develop unstable plaques and subsequent rupture, while females tend to develop more stable plaques but exhibit worse outcomes post-stroke. Despite these differences however, there is currently a lack of sex-specific guidelines for carotid atherosclerotic disease management.

Objective: To investigate the association between endogenous sex hormones and the sex-specific cellular and genetic signatures linked to plaque instability.

Methods: Carotid plaque specimens and pre-operative fasted blood samples were collected and analyzed from 460 individuals (mean age 71Â±9; 68.9% male) who underwent carotid endarterectomy at McGill University-affiliated hospitals. Sex hormones in sera were measured using LC-MS/MS to quantify 17ÃŸ-estradiol (E2) and testosterone (T). Single-cell RNA sequencing (scRNA-seq) was performed on plaque suspensions (n=10) to identify distinct cell subpopulation clusters and characterize differences in plaque cell expression phenotypes.

Results: Males with unstable plaques had significantly higher circulating T levels and T to E2 ratios compared to males with stable plaques, with no differences in E2 levels across groups. Furthermore, scRNA-seq of plaque samples revealed 15 cell subpopulations; 6 non-immune cell clusters (smooth muscle cells [SMCs], myofibroblasts, and endothelial cells) and 9 immune cell clusters (macrophages, T-lymphocytes, B-lymphocytes, natural killer cells, and dendritic

cells). In both sexes, stable plaques exhibited the highest abundance of myofibroblast-like SMCs and pro-fibrotic genes compared to unstable plaques. Similarly, female stable plaques exhibited significantly reduced number of myofibroblast-like SMCs compared to male stable atherosclerotic plaques.

Conclusions: Our findings demonstrate that taken together, increased circulating testosterone levels and reduced myofibroblast-like SMCs expressing pro-fibrotic genes in the plaque may be associated with increased carotid atherosclerotic plaque instability.

O4 CHARACTERIZATION OF PHYSIOCHEMICAL PROPERTIES AND EFFICACY OF NEW VARIANTS OF AN P3R99 ANTI-ATHEROSCLEROSIS MONOCLONAL ANTIBODY DERIVED FROM CHO-K1 AND HEK293 CELL LINES.

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Introduction: Atherosclerosis is initiated by the retention of ApoB-containing lipoproteins within the arterial wall, mediated by glycosaminoglycan chains of proteoglycans. The Center for Molecular Immunology (CIM), has developed a monoclonal antibody (mAb) (P3R99) to target this process. CIM recently produced new P3R99 mAb variants expressed in CHO-K1 and HEK-293 cell lines in order to move towards serum free media systems for phase 1 clinical trials. The purpose of this study was twofold: 1) to compare and contrast the physiochemical properties of the new variants with the original NS0 cell line to validate the potential for efficacy, and 2) Use rat and rabbit models to test biodistribution of the new variants and the capacity to interfere with atherogenesis in vivo.

Methods and Results: We compared these variants with the parental mAb from NS0 cells using SDS-PAGE, size exclusion and cation exchange chromatography, dynamic light scattering, peptide mapping, far-UV circular dichroism, and PNGase F deglycosylation. All variants exhibited a molecular size of ~150 kDa, ~99% purity, and similar average particle sizes (12.5-13.7 nm). They displayed a high β-sheet content (>40%) and basic amino acids on the surface, with minor differences in peptide maps compared to the parental mAb. Notable differences were found in the content of acidic and basic species and glycosylation profiles. NS0-derived P3R99 had lower G0F content (10.39%), higher G1F (38.29%) and G2F (30.44%) levels, with more terminal galactose (83.07%) and sialylation (15.33%). In contrast, CHO-K1 and HEK-293 variants showed similar glycosylation patterns. Despite these differences, the antigen and atherosclerotic lesion recognition properties of the mAb were unaffected in vitro. Biodistribution studies in Sprague Dawley rats (1 mg IV, n=3) revealed preferential accumulation of the new P3R99 variants in aortas and reduced LDL arterial retention (4 mg/kg, IP). Passive administration of the mAbs (2 mg every three days, three IV doses, n=6-7) in a Lipofundin 20%-induced atherosclerosis NZW rabbit model also demonstrated preferential accumulation in aortas and reduced atherosclerosis, with 60% of treated rabbits not developing lesions.

Conclusions: These results suggest that the P3R99 mAb derived from CHO-K1 and HEK-293 cells retains its antiatherogenic properties despite post-translational structural differences from the NS0-derived mAb associated with the different expression systems.

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O5 MAPPING CELL PHENOTYPES AND MICROENVIRONMENT IN HUMAN ATHEROSCLEROTIC LESIONS USING MULTIPLEX IMAGING.

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UBC

BACKGROUND: In the past few years, animal models and cultured cells have shown that smooth muscle cells (SMCs) and inflammatory cytokines are important players in atherosclerosis. Validating their roles in human atherogenesis is crucial for developing therapeutics affecting these components. Multiplex imaging is a powerful tool for mapping cell phenotypes and microenvironment in tissue sections, enabling proof-of-concept studies using biobanked human specimens. However, this technology has never been applied to human coronary arteries. This study benchmarks the first in-the-field application of the multiplex imaging platform PhenoCycler to biobanked human coronary lesions.

HYPOTHESIS: Multiplex imaging can prove theories raised by previous studies: 1. SMCs initiate foam cell formation and 2. Inflammation is associated with phenotypic switching in SMCs.

METHODS AND RESULTS: We created a 40-plex imaging panel to map lesion cells previously identified as key players in atherogenesis, including foam cells from SMC and leukocyte origins, CD68+ SMCs, apoptotic cells, and cells expressing inflammatory cytokines interleukin-1β and tumor necrosis factor α. They were imaged simultaneously on each section of early human lesions (n=15) to test theories related to foam cell formation, phenotypic transition, and inflammatory cytokines. Foam cells of SMC origin greatly outnumbered those of leukocyte origin. They enriched the deep intima, where lipids accumulate in early atherogenesis, distinct from leukocyte foam cells in the superficial intima. A higher presence of CD68+ SMCs was observed among lesion SMCs expressing high levels of interleukin-1β, but not TNF-α. SMCs and leukocytes activated different signaling pathways under similar levels of inflammation.

CONCLUSION: Our multiplex imaging results confirmed that SMCs form the majority of foam cells in early atherogenesis, and their phenotypic transition to CD68+ phenotype is related to specific inflammatory cytokines. Applying multiplex imaging to biobanked human lesions unlocks opportunities to prove that theories based on animal models and cultured cells apply to human atherogenesis.

O6 FOLLISTATIN IMPROVES VASCULAR FUNCTION BY REGULATING PERIVASCULAR ADIPOSE TISSUE IN ESSENTIAL HYPERTENSION

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Background: Essential hypertension is characterized by resistance artery remodeling induced by oxidative stress. Vessels are surrounded by perivascular adipose tissue (PVAT) which regulates vascular tone via release of vasoactive substances. In physiological conditions, PVAT induces an anticontractile effect. In pathological conditions such as hypertension, PVAT exhibit increased oxidative stress resulting in augmented contraction and reduced relaxation. Follistatin, an antioxidant glycoprotein, has been shown to lower BP in secondary hypertension. Follistatin induces adipose tissue browning which is linked to improved vasorelaxation and blood pressure (BP).

Hypothesis: Follistatin, through ROS inhibition and PVAT browning, lowers BP and improves vessel function in a model of essential hypertension.

Methods: Spontaneously hypertensive rats (SHR) were treated with vehicle or follistatin every other day for 8 weeks. Wistar Kyoto (WKY) rats served as normotensive controls. BP was measured weekly by radiotelemetry. Mesenteric arteries and PVAT were used. KCl-induced smooth muscle contraction was assessed using wire myography. ROS was measured by dihydroethidium (DHE) staining. Nitric oxide (NO) bioavailability in PVAT was measured using DAF-2 FM. Markers for ROS or PVAT browning was measured by immunohistochemistry. Ex vivo PVAT browning was assessed in isolated PVAT treated with follistatin for 3 days.

Results: Follistatin significantly lowered BP in SHRs and restored anticontractile effect of PVAT. Follistatin inhibited SHR PVAT-induced vascular oxidative stress. NO bioavailability in PVAT was increased by acute follistatin treatment. Follistatin also increased expression of brown adipose tissue markers in SHR PVAT suggesting PVAT browning. Acute follistatin induces PVAT browning.

Conclusions: Follistatin lowers BP in SHRs likely via improved vascular and perivascular structure and function. Specifically, follistatin inhibited perivascular ROS, increased NO and induced PVAT browning suggesting improved regulation of vascular function and thus, BP. Future work will utilize proteomic assays to identify novel mediators of follistatin effects on PVAT in the SHR.

O7 PNEUMONIA INDUCES LONG-TERM CHANGES IN IMMUNE CELLS IN ATHEROSCLEROSIS

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Background: Pneumonia is inflammation of the lungs caused by an infection. Pneumonia patients present with a 4x and 2x higher risk of a cardiac event within the first 30-days and 10 years, respectively, following hospitalization. Moreover, pneumonia survivors present with higher inflammatory activity in the lungs and blood vessels 30-45 days following hospitalization. As such, it is possible that the higher inflammatory burden in this patient population is driving the progression of atherosclerotic vascular disease.

Hypothesis: Pneumonia-associated inflammation causes long-lasting immune imprinting in hematopoietic stem cells and mature immune cells that exacerbates atherosclerosis progression.

Methods: We have developed a dual-disease mouse model of pneumonia using Streptococcus pneumoniae (a well-known model of bacterial pneumonia) and atherosclerosis (Apoe-/-). Mice were placed on a western diet for 8-weeks, followed by a return to chow diet for 4-weeks, prior to intranasal inoculation with S.pneumoniae (10^4 CFU) or PBS. Tissue collection and analyses were performed 28-days following inoculation.

Results: Mice inoculated with S.pneumoniae experienced a high mortality rate with 50% of females and 30% of males surviving to endpoint analysis (compared to 80% on chow diet). We observed a higher number of neutrophils in the lungs (1.46x higher, p=0.0484) and blood (1.45x, p=0.0035) in mice recovered from S.pneumoniae pneumonia compared to PBS. Moreover, we observed a significant increase in the number of neutrophils (1.45x, p=0.0002) and granulocyte and monocyte progenitors (1.29x, p=0.0168) in the bone marrow of mice recovered from S.pneumonaie pneumonia. Importantly, there was no S.pneumoniae bacterial colonies detected in the lungs, blood, and spleen.

Conclusion: There remains persistently elevated markers of inflammation in mice with established atherosclerosis inoculated with S.pneumoniae compared to PBS. These immune cells appear to be derived from the bone marrow niche. Next steps include measuring atherosclerotic burden.

O8 REGULATION OF GUT MICROBIOTA BY D-MANNOSE IS ASSOCIATED WITH REDUCED ATHEROSCLEROSIS IN FEMALE APOE-/- MICE.

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Background: D-mannose, a glucose epimer involved in protein glycosylation, is found throughout the body and has been shown to influence gut microbiota and reduce obesity in mice on a high-fat diet. Its effects on atherosclerosis are not well understood.

Hypothesis: D-mannose supplementation might counteract high fat diet (HFD) induced atherosclerosis by regulating gut microbiota.

Methods & results: ApoE-/- mice were fed a chow diet (CD) or HFD for 9 weeks with access to water containing 0%, 5%, or 20% D-mannose. Body weight, lipid levels, and glucose tolerance showed no significant differences among groups. However, atherosclerotic plaque burden was significantly reduced in the aortic sinus (0.29±0.02 (5%), 0.33±0.03 (20%) vs 0.47±0.04 (0%) mm2) and brachiocephalic artery (0.059±0.01 (5%), 0.051±0.013 (20%) vs 0.082±0.009 (0%) mm2) in mice receiving 5% and 20% mannose compared to controls. Gut microbiota 16S sequencing indicated that mannose supplementation also altered the intestinal microbiome. HFD increased the Firmicutes/Bacteroidetes ratio by 2.7-fold compared with chow (6.1±1.5 vs. 2.0±0.3), which was prevented by 20% mannose (2.5±0.1). Furthermore, there was a significant correlation between Lactobacillaceae abundance and plaque size ($R2=0.32$, $P<0.01$). Antibiotic treatment prevented the protective effect of mannose. However, fecal microbiota transplantation with 20% mannose-treated mice feces did not reduce plaque size in HFD-treated ApoE-/- mice (0.040±0.020 (20%) vs 0.054±0.02 (0%) mm2). Additionally, 5 and 20% mannose reduced gene expression levels of IL-17A, IL-6, and TNF-alpha, gut pro-inflammatory cytokines. Gut microbiota 16S sequencing indicated that mannose supplementation also altered the intestinal microbiome. HFD increased Firmicutes/Bacteroidetes ratio by 2.7-fold compared with chow (6.1±1.5 vs. 2.0±0.3), which was prevented by 20% mannose (2.5±0.1). Furthermore, there was a significant correlation between Lactobacillaceae abundance and plaque size ($R2=0.32$, $P<0.01$). Antibiotic treatment prevented the protective effect of mannose. However, fecal microbiota transplantation with 20% mannose-treated mice feces did not reduce plaque size in HFD-treated ApoE-/- mice (0.040±0.020 (20%) vs 0.054±0.02 (0%) mm2). Additionally, 5 and 20% mannose reduced gene expression levels of IL-17A, IL-6, and TNF-alpha, gut pro-inflammatory cytokines.

Conclusion: Our results demonstrate that oral mannose supplementation exerts a protective effect in ApoE-/- mice fed a HFD, by reducing atherosclerotic lesion development. Our data further demonstrate that the gut microbiota plays an important role in regulating plaque formation, and that the beneficial effects of mannose supplementation may be at least partly related to its ability to regulate gut microbiota composition.

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O9 INVESTIGATING THE ROLE OF INTERLEUKIN-15 RECEPTOR ALPHA IN ATHEROSCLEROSIS

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Background: Atherosclerosis is a chronic inflammatory disease driven by lipid accumulation and macrophage activation that induce cytokine production. Interleukin-15 (IL-15) and its receptor, IL-15Rα, have been detected in murine and human atherosclerotic plaques but little is known about the effect of IL-15Rα on atherosclerosis.

Hypothesis: Genetic inactivation of IL-15Rα protects against atherosclerosis development.

Methods and Results: Spontaneous and diet- accelerated atherosclerosis were analyzed in IL-15Rα-/- ApoE-/- (dKO) and control ApoE-/- mice by feeding mice a normal diet and a high-fat diet(HFD) respectively. The dKO mice were significantly protected against atherosclerosis development when compared to ApoE-/- mice, irrespective of the diet used. Parabiosis (surgical connection of circulatory systems of mice) was used to determine if the observed reduction in atherosclerosis was due to the absence of IL-15Rα expression locally in the vessels developing atherosclerotic plaques. Parabiosis between ApoE-/- and the dKO mice dramatically increased atherosclerosis in the dKO mice whereas atherosclerosis levels in the ApoE-/- mice were unaffected. This suggests that the reduction in atherosclerosis development in dKO mice is due to the absence of circulating factor(s), that could be supplied in trans from the circulation of ApoE-/- mice expressing IL-15Rα. HFD- accelerated atherosclerosis was also analysed in IL-15-/- ApoE-/- mice and was found to be unaffected when compared to ApoE-/- mice. Interestingly, deleting IL-15Rα in the IL-15-/- ApoE-/- mice significantly reduced atherosclerosis development in the mice, suggesting that the protection against HFD-induced atherosclerosis, conferred by IL-15Rα deletion, was independent of its only known ligand, IL-15.

Conclusion: IL-15Rα deletion protects against atherosclerosis development in ApoE-/- mice, independently of IL-15. The protection results from the absence of one or more circulating factor(s) that would normally drive atherosclerosis development. The identification of the circulating factor(s) that drive atherosclerosis may lead to novel insights into mechanisms of atherosclerosis development.

O10 LIPOPROTEIN(A) AND THE ARTERIAL ENDOTHELIUM: ELUCIDATING MECHANISMS OF RESIDUAL CARDIOVASCULAR RISK

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BACKGROUND: Atherosclerosis is initiated by deposition of lipids, particularly low-density lipoprotein (LDL), underneath the arterial endothelium which occurs via a form of vesicular traffic known as transcytosis. Transcytosis of LDL is performed by receptors and vesicular structures called caveolae, characterized by the integral membrane protein caveolin-1 (Cav-1). Despite effective treatments to lower LDL levels, patients still experience cardiovascular events, suggesting that residual risk factors exist. Lipoprotein(a) [Lp(a)] is a lipoprotein

structurally similar to LDL that is significantly more atherogenic. Lp(a) can bind to oxidized phospholipids, inducing inflammation, which then exacerbates its pro-atherogenic effects. Although Lp(a) is a known cardiovascular risk factor, the mechanisms by which it promotes atherosclerosis are not yet understood.

HYPOTHESIS: 1)Since Lp(a) is structurally like LDL, Lp(a) may undergo endothelial transcytosis and accumulate in the vessel wall. 2)Considering the role of Lp(a) in inflammation, Lp(a) may stimulate endothelial LDL transcytosis.

METHODS AND RESULTS: 1)Lp(a) transcytosis: Using Total Internal Reflection Fluorescence (TIRF) microscopy and Human Coronary Artery Endothelial Cells (HCAECs), Lp(a) was discovered to undergo transcytosis at a lower rate than LDL. However, internalization of Lp(a) was similar to LDL, suggesting a more efficient process. Saturation of Lp(a) transcytosis in female compared to male cells was observed. Lp(a) transcytosis is receptor-mediated and appears to compete with LDL for the same transcytosis receptors. Knockdown of caveolin-1 using siRNA reduced Lp(a) transcytosis substantially. Acute, dose-dependent deposition of Lp(a), but not equimolar dextran (control for paracellular leakage), was measured in atheroprone region of the murine aortas. 2)Lp(a)-stimulated LDL transcytosis: Notably, treating HCAECs with Lp(a) for 24hrs, significantly stimulated LDL transcytosis.

CONCLUSIONS: Lp(a) can undergo receptor-mediated endothelial transcytosis and can stimulate LDL transcytosis. We are currently determining the molecular mechanisms behind both phenomena in vitro and validating our results in wild type vs tissue-specific knockout animals.

O11 INVESTIGATING THE ROLE OF ANGIOGENESIS ON ATHEROSCLEROTIC PATHOGENESIS IN DIABETIC MURINE MODELS

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Background: Diabetes Mellitus is characterized by chronically elevated blood glucose levels with an elevated risk of developing atherosclerotic cardiovascular disease (CVD). However, the molecular mechanisms linking diabetes and atherosclerosis remains unclear. We have previously observed that accelerated atherogenesis in hyperglycemic ApoE-/- mice is associated with a decrease in vasa vasorum density in the aortic sinus and decreased vascular endothelial growth factor (VEGF) expression in the aorta wall, despite elevated levels of hypoxic markers. Hypothesis: Hyperglycemia impairs angiogenesis and contributes to vascular hypoxia, accelerating atherosclerosis development. Therefore, we hypothesis that overexpression of VEGF in the artery wall will attenuate atherosclerosis in hyperglycemic mice.

Methods: Circulating levels of systemic angiogenic factors (VEGF, Angiopoietin-2, and Endostatin) were measured by ELISA. Five-week-old male and female ApoE-/- CSF1R-iCretg/0 ROSA-VEGF164tg/0 mice were randomly assigned to experimental groups (n=8/group). Hyperglycemia was induced by multiple low dose streptozotocin injections. VEGF overexpression was induced at 6 weeks or 10 weeks of age. Blood, heart, and aorta samples were collected at 15 weeks of age for analysis.

Results: Hyperglycemic ApoE-/- mice exhibited significantly elevated systemic pro-angiogenic factors (Ang-2 and VEGF) and reduced anti-angiogenic factor (Endostatin), indicating a shift towards neovascularization. In hyperglycemic female mice, early transient VEGF overexpression (at 6 weeks of age) led to reduced atherosclerotic plaque volume and decreased expression of the hypoxic marker, HIF-1α.

Significance: This study reveals a novel mechanism by which diabetes and hyperglycemia accelerate atherosclerosis through the dysregulation of VEGF and angiogenesis.

O12 DEGRADATION-RESISTANT ADAMTS13 VARIANTS EXHIBIT IMPROVED STABILITY AND FUNCTIONAL ACTIVITY

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McMaster University

Background: Thrombosis is a pathological condition characterized by the abnormal formation of blood clots within vessels. Thrombosis is responsible for 1 in 4 deaths worldwide, yet current therapies are ineffective due to significant risks of bleeding. An emerging therapy that can treat thrombosis without causing bleeding is the administration of ADAMTS13. ADAMTS13 is a circulating protease that regulates von Willebrand Factor (VWF), a protein that causes clot formation. Preclinical studies have shown that ADAMTS13 improves outcomes in several thrombotic disorders including heart attack and stroke. However, we have found that ADAMTS13 is degraded in the blood, which reduces its antithrombotic activity. To engineer more effective ADAMTS13-based therapies, we designed a degradation-resistant ADAMTS13 variant.

Hypothesis: We hypothesize that this degradation-resistant ADAMTS13 variant can retain its ability to prevent excessive clot formation.

Methods: In silico and in vitro analyses were conducted to identify site(s) of cleavage within ADAMTS13. Substitution mutations were made to T4L and T8L domains on ADAMTS13, then cloned and expressed. Degradation-resistance was assessed by incubation with coagulation proteases and western blotting. A fluorescence resonance energy transfer assay and microfluidics flow model were used to assess ADAMTS13 activity.

Results: Reducing SDS-PAGE and Sypro-ruby staining revealed that substitutions of the T4L/T8L domain with glycine-serine linkers protected ADAMTS13 from degradation by plasmin, thrombin, kallikrein, and factor XIa. Degradation-resistant ADAMTS13 exhibits complete resistance to proteolytic cleavage from plasmin and thrombin over 180 minutes. Under static conditions, the enzymatic rate of degradation-resistant ADAMTS13 does not differ significantly from wild-type (WT) ADAMTS13. In a microfluidics flow model of the vasculature, degradation resistant-ADAMTS13 reduces VWF-platelet complexes on endothelial cell surfaces similarly to WT ADAMTS13 ($p<0.05$).

Conclusion: Our results demonstrate that degradation-resistant ADAMTS13 is resistant to coagulation and plasma protease degradation and retains its functional activity in vitro.

O13 INVESTIGATING THE OPTIMAL TIME OF ADMINISTRATION OF HEPARIN THAT WILL IMPROVE SURVIVAL IN A MURINE MODEL OF SEPSIS

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Background: Sepsis is life-threatening organ dysfunction due to a dysregulated host response to infection, leading to shock, organ failure, and death (Singer et al., 2016). A hallmark of sepsis is infection-induced thrombosis ("immunothrombosis"). Recently, there's been a renewed interest in heparin as a therapy for sepsis as it can target harmful pathways of immunothrombosis by inhibiting coagulation and inflammation. In COVID-19 clinical trials, therapeutic-dose anticoagulation using heparin shows protective effects in moderately ill, but not critically ill

patients with COVID-19-associated sepsis (The ATTACC, ACTIV-4a, and REMAP-CAP Investigators, 2021). This suggests that initiating therapeutic-dose anticoagulation late in sepsis may be too late to alter the consequences of immunothrombosis. Thus, the time of initiation of therapeutic-dose heparin may be important during sepsis.

Hypothesis: There is an optimal time of administration of heparin that will improve survival in a 48-hour fecal-induced peritonitis (FIP) model of sepsis.

Methods: A 48-hour FIP model was used to induce abdominal sepsis. Ten- to twelve-week-old C57Bl/6 mice received an intraperitoneal (IP) injection of fecal slurry. They were treated with buprenorphine, antibiotics, fluids, and either saline, or LMWH (Dalteparin, subcutaneously 100 IU/kg/12 h). We assessed 48-hour survival along with physiological parameters, blood biomarkers, organ histology, and thrombin generation.

Results: Early administration of Dalteparin (2- or 4-hours post-FIP) did not improve survival. However, administration of Dalteparin provided a significant survival benefit in septic mice when administered at 8-hours post-FIP (73.3%) compared with saline-treated mice (21.4%) ($p<0.01$). Similarly, administration of Dalteparin provided a significant survival benefit at 12-hours post-FIP (70%) compared with saline-treated mice (27.8%) ($p<0.05$).

Conclusions: Administration of Dalteparin at 8- or 12-hours post-FIP provides a significant survival benefit, whereas earlier administration does not. These findings suggest that the optimal time of administration of Dalteparin to improve survival in a murine model of sepsis is between 8- and 12-hours post-FIP.

O14 INVESTIGATING MOLECULAR CHANGES TO UNRAVEL THE CHRONOLOGY OF CARDIOVASCULAR DAMAGE IN TYPE 1 DIABETES.

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Background: Type 1 diabetes (T1D) is an autoimmune disease, often diagnosed in childhood, characterized by the destruction of insulin-producing pancreatic beta cells. Population-based studies show that individuals with T1D are 10-times more likely to develop cardiovascular disease. Vascular damage is present in children with T1D and includes endothelial dysfunction, arterial stiffness, and greater carotid intima-media thickness (cIMT). However, the chronology and pathophysiology of vascular damage in T1D are not fully understood. This study aims to investigate molecular mechanisms and chronology of vascular damage during pediatric diabetes progression to identify biomarkers of early vascular damage.

Hypothesis: A pathological pattern of vascular gene expression and biomarkers of vascular damage will be present at diabetes onset and will worsen over time.

Methods: Studies were conducted in C57BL/6J-Ins2Akita (Ins2+/Akita) mice, as a model of pediatric diabetes, and their Ins2+/+ littermates. Single-cell RNA sequencing (scRNA-seq) was conducted on the aorta of male and female Ins2+/Akita and age-matched control mice (Ins2+/+ littermates) at: 1) diabetes onset (blood glucose ≥16.7mmol/L); and 2) 4-weeks post-diabetes onset. Raw scRNA-seq data was processed and analyzed using Cell Ranger (10X Genomics) and the R package Seurat. Differential gene expression patterns will be confirmed by immunohistochemical analysis. Serum will be analyzed for biomarkers of vascular damage and inflammation.

Results: Clustering analysis of transcriptional profiles of 55,733 aortic cells identified 34 clusters representing main cell types: endothelial cells, smooth muscle cells, fibroblasts, immune cells (T-cells, B-cells, and macrophages), mesothelial cells and neural cells. Preliminary analysis revealed reduced immune cell populations in Ins2+/Akita male mice

versus age-matched controls at the onset of diabetes and 4-weeks post-diabetes onset. Further analysis of cell-specific differential gene expression is underway.

Conclusions: Investigating changes in cellular gene expression in aorta during progression of diabetes may uncover markers of vascular damage that can be targeted for prevention and treatment of diabetes-associated cardiovascular complications

O15 THE EFFECTS OF CHRONIC PSILOCYBIN ADMINISTRATION IN HIGH FAT DIET-FED MALE AND FEMALE C57BL/6J MICE

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Background: Obesity affects 1 in 4 Canadians. Overeating, as a contributor to obesity, shares neurobiological and environmental parallels with other addictive disorders. Psilocybin, a psychoactive compound in Psilocybe mushrooms, has shown promise in treating addiction disorders. Although currently restricted in Canada, this has led to an interest in psilocybin for obesity treatment. Therefore, we investigated the physiological, metabolic and behavioural effects of chronic psilocybin administration in female and male mice challenged with a high-fat diet (HFD).

Hypothesis: Psilocybin will attenuate weight gain in male and female mice fed a HFD, but not a low-fat diet (LFD).

Methods: Eight-week-old female and male C57BL/6J mice were randomized to a 45 kcal% HFD or 10 kcal% LFD for 17 weeks, and further randomized to receive weekly i.p. injections of 1 mg/kg psilocybin or vehicle (isotonic saline) for an additional 19-weeks. Mice were subjected to open field testing (OFT), respiratory gas exchange and movement assessment, voluntary activity assessment, glucose tolerance (GTT) and insulin sensitivity testing (ITT), and were monitored for food intakes and body weights.

Results: HFD-induced weight gain was not attenuated by psilocybin in either sex. In the LFD group, male and female mice treated with psilocybin had significantly lower body weights than vehicle-treated controls, however, food intakes did not differ. OFT demonstrated that males fed a HFD experienced significant thigmotaxis, decreased velocity and distance traveled, but these effects were not corrected by psilocybin. Psilocybin did not alter glucose tolerance compared to saline within sex- and diet-matched groups. However, blood glucose was significantly lower in male-LFD mice given psilocybin, compared to saline-treated controls after a brief (2h) fast (6.00 +/- 0.37 versus 8.58 +/- 0.94 mM, respectively).

Conclusion: Psilocybin showed a minor benefit in attenuating age-related weight gain in LFD male and female mice, associated with evidence of metabolic improvement.

O16 LOSS OF TDAG51 ATTENUATES METHIONINE AND CHOLINE DEFICIENT DIET-INDUCED ER STRESS AND LIVER INJURY

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BACKGROUND: T-cell death associated gene 51 (TDAG51) also known as the pleckstrin homology-like domain family A member 1 (PHLDA1) is an endoplasmic reticulum (ER) stress responsive gene with implications in cardiometabolic disease. Previously, it was reported that

whole-body deletion of TDAG51 is associated with obesity, insulin resistance and hepatic steatosis in mice. In this study, we aimed to investigate the local effect of liver-specific TDAG51 deletion under conditions of methionine and choline deficient (MCD) diet-induced ER stress.

HYPOTHESIS: We hypothesized that the loss of hepatic TDAG51 levels will cause increased hepatic lipid accumulation and delay liver fibrosis due to impaired ER stress signalling.

METHODS AND RESULTS: The effects of liver-specific deletion of TDAG51 was examined in a six week MCD diet model, commonly used to induce hepatic inflammation, apoptosis, and fibrosis. Liver-specific loss of TDAG51 protein levels increased adiposity, serum triglycerides and hepatic steatosis as measured by and Oil red O quantification. Loss of hepatic TDAG51 protein resulted in decreased markers of the unfolded protein response, fibrosis and apoptosis. In particular, MCD-fed liver-specific TDAG51-null mice demonstrate significantly reduced GRP78 protein, a molecular chaperone in the ER and master regulator of the UPR. Consistent with an observed reduction in histopathological staining of liver fibrosis, serum TGF-beta levels were significantly lowered in liver-specific TDAG51-null mice compared to controls. Furthermore, loss of hepatic TDAG51 protein reduced the liver injury marker, ALT, compared to controls under MCD-fed conditions. In line with these observations, liver-specific TDAG51-null mice demonstrate reduced TUNEL staining, an indication of reduced cell death.

CONCLUSIONS: These findings suggest that PHLDA1/TDAG51 is a potent regulator of the UPR and mediates downstream ER stress-induced fibrosis and apoptosis. Therapeutic strategies aimed at modulating hepatic levels of PHLDA1/TDAG51 to attenuate hepatic ER stress and chronic UPR activation represent a novel treatment approach for MAFLD.

O17 UNVEILING THE INTERPLAY OF DPP4 AND EPDR1 IN OBESITY: IMPLICATIONS FOR INFLAMMATION AND ATHEROSCLEROSIS

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Background: Approximately 27% of adults in Canada are living with obesity which greatly increases the risk of developing cardiometabolic diseases like atherosclerosis. Studies have shown increased expression of dipeptidyl peptidase 4 (DPP4) on monocytes and macrophages (which are critical players in the pathogenesis of atherosclerosis) and increased plasma DPP4 concentrations are associated with clinical measurements of atherosclerosis and metabolic dysfunction. DPP4 is a serine protease that regulates the bioactivity of several peptide substrates. Our group identified mammalian ependymin-related protein 1 (EPDR1) as a novel substrate for DPP4. Atherosclerotic genetic signatures have implicated EPDR1 in cardiometabolic disease progression and found that EDPR1 reduced glucose and lipid risk factors, suggesting EPDR1 is cardioprotective. However, the source of EPDR1, its target tissues, and whether DPP4-mediated cleavage results in a reduction of its bioactivity, is not yet known.

Hypothesis: DPP4 regulates the bioactivity of EPDR1 in circulation and on macrophages to drive inflammation and atherosclerosis progression.

Methods and Results: After polarization of bone marrow-derived macrophages with LPS+IFNγ (pro-inflammatory) for 48 hours, we observed a 5.5-fold increase in Dpp4 mRNA and a 5-fold increase in cellular Dpp4 protein concentration compared to IL4-treated (pro-resolving) macrophages. We have also identified a 3-fold increase in EDPR1 gene expression in pro-resolving macrophages. The Hybrid-Mouse Diversity Panel (HMDP) characterized over 100 in-bred strains of mice to analyze the association of genetic and environmental factors for

metabolic disease progression. Upon 8-weeks of a high-fat/high-sugar diet, we found that Epdr1 expression in perigonadal white adipose tissue is positively correlated with fat mass, glucose and insulin levels, and insulin resistance. Epdr1 expression on macrophages is positively correlated with total fat mass suggesting links between lipid accumulation and inflammation with EPDR1.

Conclusions: These results implicate a possibility for DPP4 in regulating EPDR1 in states of inflammation and atherosclerotic progression. We hypothesize that up-regulation of EDPR1 is through lipid accumulation as a homeostatic mechanism to possibly prevent further disease progression.

O18 THE ROLE OF MEMBRANE TYPE 1- MATRIX METALLOPROTEINASE IN THE DEVELOPMENT OF LIVER FIBROSIS

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BACKGROUND: Liver fibrosis is a chronic liver disease that occurs due to continuous extracellular matrix (ECM) remodeling leading to deposition of fibrous scar tissues in the liver. Advanced liver fibrosis is a severe global health problem recognized as the most reliable predictor of liver related mortality. Membrane type 1- matrix metalloproteinase (MT1-MMP/MMP14), is a cell membrane-tethered endopeptidase well studied for its role in the cleavage of ECM proteins. It is the only MMP that the embryonic deletion leads to death in mice at 3-4 weeks after birth, making it crucial to several physiological processes.

HYPOTHESIS: Despite the well established role of MT1-MMP in the remodeling of the ECM, no study has shown the role of MT1-MMP in the development of liver fibrosis. Therefore, this study aims to investigate the role of MT1-MMP in the development of liver fibrosis.

METHODS AND RESULTS: MMP14 expression was blunted with siRNAs in LX2 cells, an immortalized human hepatic stellate cells (HSC), to study the effect on activation of HSC. Liver fibrosis was induced in hepatocyte (Mmp14HepKO) and HSCs (Mmp14HSCKO) specific Mmp14 knockout mice models, using chemical and diet models. MMP14 knockdown in LX2 cells caused significant intracellular accumulation of collagen, and fibronectin. After inducement of liver fibrosis, Mmp14HSCKO but not Mmp14HepKO showed more collagen accumulation stained by picro sirus red, compared to Mmp14fl/fl mice. Hepatic levels of alpha smooth muscle actin, and interleukin 1β were significantly higher in the Mmp14HSCKO compared to either Mmp14fl/fl or Mmp14HepKO. Partial knockout of MMP14 in the HSCs (HSC-Mmp14+/-) accumulated higher collagen than Mmp14fl/fl but lower than the homozygous Mmp14HSCKO. At 30 weeks of NASH-diet feeding, Mmp14HSCKO but not Mmp14fl/fl developed hepatocellular carcinoma and a profound loss of visceral fat accumulation.

CONCLUSIONS: This study has shown that MMP14 in the HSCs play a crucial role in the development and progression of liver fibrosis.

Abstracts of Poster Presentations

P1. ACCELERATED PROGRESSION OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN YOUNG WOMEN WITH POLYCYSTIC OVARY SYNDROME

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BACKGROUND: Polycystic Ovary Syndrome (PCOS) is the most prevalent endocrine-metabolic disorder, affecting approximately 10-15% of women across the lifespan. Women with PCOS are at increased risk of obesity, diabetes and cardiovascular disease (CVD). We have previously shown high-risk overweight-obese women with and without PCOS exhibit atherogenic dyslipidemia, with elevated TG, apoB48, and remnant-cholesterol (RC) compared to healthy-weight controls. Those with PCOS have increased atherosclerotic cardiovascular disease (ACVD): higher carotid plaque height (CPH) and carotid intimal medial thickness (cIMT) compared to healthy-weight controls. The aim of this study was to examine progression of atherogenic dyslipidemia and ACVD in a cohort of young women with and without PCOS at baseline and at 2 years follow up.

METHODS: A cohort of overweight-obese (BMI >25kg/m2) females aged 18-45 years with and without PCOS matched for age-BMI, and healthy-weight controls were assessed at baseline and at 2 years follow up. Measurements included blood lipids and apoB (apoB48 & apoB100), remnant-cholesterol, hormones, insulin-glucose, and ACVD (CPH, cIMT) using 2D and 3D-echocardiography.

RESULTS: To date, 23 PCOS, 6 non-PCOS controls and 7 healthy-weight controls have completed follow up testing, with a mean follow-up duration 2.6 years. Our preliminary data shows lipid and hormone profile had similar trends to baseline data across groups. TG and RC were 2-fold higher in PCOS compared to non-PCOS controls, and 2.5-fold higher compared to healthy-weight controls. Non-HDL-C was 40% lower in both PCOS and non-PCOS controls compared to healthy-weight controls. Total ApoB was 16% higher in PCOS compared to non-PCOS and healthy-weight controls. cIMT increased from baseline by 7.9% in PCOS, compared to 6.4% in non-PCOS and 4.9% in healthy-weight controls. Of those who had carotid plaque at baseline, CPH increased 6-15 fold in those with PCOS compared to controls at follow-up.

CONCLUSION: Our preliminary follow-up data shows that high-risk young women with PCOS have exacerbated CVD risk factors and accelerated progression of ACVD compared to age-BMI matched and healthy-weight controls.

P2. HDL ATTENUATES ANG II-AT1R-EGFR SIGNALING AND REVERSES VASCULAR REMODELING IN SPONTANEOUSLY HYPERTENSIVE RATS

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Background: Angiotensin II (Ang II) signaling via angiotensin II type 1 receptor (AT1R) and transactivation of epidermal growth factor receptor (EGFR) enhanced VSMCs proliferation contributing to vascular remodeling evident in spontaneously hypertensive rats (SHR) aorta. High-density lipoprotein (HDL) reduced blood pressure in SHR, yet via elusive mechanism(s). We propose that HDL attenuates Ang II-AT1R-EGFR signaling and reverses vascular remodeling in SHR.

Methods: Wistar Kyoto rats (WKY) and SHR were treated with HDL in vivo for one week. Vascular remodeling was histologically examined. VSMCs proliferation and the expression levels of AT1R, EGFR, extracellular signal regulated kinases 1/2 (ERK1/2), scavenger receptor

class B type-I (SR-BI) and its adaptor protein PDZK1 were examined by immunofluorescence. VSMCs proliferation was further examined in vitro.

Results: In vivo HDL treatment reduced blood pressure in SHR, increased aortic lumen diameter, reduced media thickness to lumen diameter ratio and decreased collagen contents in SHR. Furthermore, HDL treatment decreased the number of proliferating VSMCs and α-smooth muscle cell actin, reduced the expression of AT1R and EGFR and increased the expression of SR-BI adaptor protein, PDZK1, in SHR aorta. In isolated VSMCs, HDL attenuated Ang II-induced proliferation by reducing AT1R expression and decreasing Ang II-induced transactivation of EGFR. HDL effects were SR-BI dependent and were mimicked by different HDL subpopulations, reconstituted HDL, and lipid free apolipoprotein A-I.

Conclusions: HDL attenuates Ang II-AT1R-EGFR signaling, reduces VSMCs proliferation, and reverses vascular remodeling in SHR. HDL modulation of vascular remodeling could be one mechanism by which HDL reduces blood pressure in SHR.

P3 CHARACTERIZATION OF PLAAT1-/- MICE

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BACKGROUND: We have previously found that phospholipase A and acyltransferase 1 (PLAAT1) catalyzes the synthesis in vitro of cardiolipin using monolysocardiolipin as an acyl acceptor and phosphatidylcholine as an acyl donor. This enzyme also catalyzes N-acyltransferase and O-acyltransferase reactions, which are involved in the synthesis of signaling lipids including the endocannabinoids. These bioactive lipids also play roles in regulating cardiolipin metabolism, and therefore loss of this enzyme may affect tissue cardiolipin indirectly as well. To investigate the physiological role of this enzyme, we have created Plaat1 total knockout mice (Plaat1-/-).

HYPOTHESIS: We hypothesized that loss of Plaat1 would result in reduced cardiac cardiolipin content.

METHODS and RESULTS: Female and male mice were studied. Body weights were indistinguishable between male wildtype and Plaat1-/-mice, but food intakes were lower and heart and gastrocnemius muscle depots weighed less in knockouts. Female mice also had smaller hearts, but no other significant differences in body or organ weights, or food intakes. Male and female Plaat1-/- heart tissue contained ~1/3rd less cardiolipin compared to their sex-matched controls, while cardiolipin linoleic acid concentrations were >40% lower, as determined using gas chromatography. Levels of mitochondrial marker proteins in hearts from Plaat1-/- mice were largely similar, although a slight reduction in SDHA levels were detected. Total daily oxygen consumption, carbon dioxide production, and energy expenditure, as measured using the Comprehensive Laboratory Animal Monitoring System, were significantly lower in male and female Plaat1-/-mice, and males had lower rearing activity. Plaat1-/- exercise capacity, assessed using a treadmill test, was slightly but significantly impaired.

CONCLUSIONS: PLAAT1 is a novel enzyme in cardiolipin metabolism required for exercise tolerance, normal respiration, and the maintenance of cardiac cardiolipin content.

P4 DEEP PROTEASE PROFILING DECONVOLUTES PROTEASE SIGNATURES RELEASED FROM ACTIVATED NEUTROPHILS

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INTRODUCTION: Dysregulated protease activity is a key contributor to pathogenesis and progression of conditions like cancer, neurodegenerative, and thromboinflammatory diseases. However, detecting protease activity in complex biological mixtures is limited, because current substrate-based probes may lack sufficient specificity and require prior knowledge of protease content to select probes.

OBJECTIVES: To develop Deep Protease Profiling as a method for unbiased characterization of protease activity in mixtures and validate its use with activated neutrophil supernatants.

METHODS: A substrate phage-display library of nearly every possible 5-amino acid peptide was engineered. Libraries were screened against activated neutrophils and 3 purified neutrophil serine proteases (elastase, cathepsin-G, human proteinase-3). Cleaved phages underwent high-throughput sequencing.

RESULTS: Identified substrate specificities for purified neutrophil serine proteases aligned with existing literature, prior protease screens, and known active site architecture. Proteolytic activity in activated neutrophil supernatants was identified to be primarily from serine proteases. Reference matrices from purified protease datasets were next applied to the activated neutrophil supernatant using 2 bulk RNASeq deconvolution algorithms (CibersortX and EPIC). Both CibersortX and EPIC identified the 3 neutrophil serine proteases in the activated neutrophil supernatant. Importantly, signatures for thrombin and ADAMTS13 reference matrices were not found in the activated neutrophil datasets.

CONCLUSION: We demonstrated an unbiased and systematic method to detect and deconvolute protease activity in complex biological mixtures. Future studies will expand Deep Protease Profiling to other proteases, mixtures, and clinical samples, with promise as an emerging platform for developing novel diagnostic tools to manage disease in patients.

P5 EPA AND DHA ATTENUATE, BUT DOES NOT ELIMINATE, THE ASSOCIATION OF PLASMA APOB-TO-PCSK9 RATIO TO METABOLIC RISK IN HUMANS

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BACKGROUND: Elevated plasma numbers of apoB-lipoproteins (i.e. apoB) promote white adipose tissue (WAT) dysfunction, inflammation and related risk for type 2 diabetes (T2D). We reported that plasma apoB-to-PCSK9 ratio is a better predictor of T2D risk factors than plasma apoB as it predicts higher WAT-surface expression of LDL receptors (LDLR and CD36). Recently, we showed that 3-month supplementation with eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) increased % EPA+DHA in plasma phospholipids (omega-3 index) and eliminated group-difference between subjects with high versus low plasma apoB in WAT inflammation related to the NLRP3 inflammasome/IL-1β pathway.

HYPOTHESIS: 3-month supplementation with EPA and DHA eliminates the association of plasma apoB-to-PCSK9 ratio to T2D risk factors.

METHODS and RESULTS: Post-hoc analysis was conducted on subjects who completed the 3-month trial with 2.7 gm/day EPA and DHA (N=33, BMI=29.7±1.0 kg/m2, age=58.7±1.4 years) (NCT04496154). Insulin secretion and sensitivity were measured by Botnia clamps, WAT surface-expression of LDLR and CD36 by immunochemistry, WAT gene expression by RT-qPCR, plasma PCSK9 by ELISA kits. At baseline, fasting plasma apoB-to-PCSK9 ratio correlated positively with WAT-surface expression of LDLR and CD36 (r=0.45 and r=0.62, respectively) but negatively with their mRNA expression levels. It also correlated positively with WAT NLRP3 and IL1B expression, postprandial hypertriglyceridemia and 1st and 2nd phase insulin secretion (r=0.39 to 0.45), and negatively with WAT ADIPOQ and SREBP1 expression (markers of WAT function) and insulin sensitivity (M/Iclamp) (r=-0.36 to -0.38). EPA and DHA supplementation improved β-cell function, decreased postprandial hypertriglyceridemia and plasma PCSK9. Post-intervention plasma apoB-to-PCSK9 remained associated with T2D risk factors, except for 2nd phase insulin secretion, M/Iclamp and WAT surface-expression of LDLR.

CONCLUSION: EPA and DHA attenuate, but does not eliminate, the association of plasma apoB-to-PCSK9 with T2D risk factors. This ratio remains a better index of metabolic risk than plasma apoB independent of the omega-3 index.

P6 ENERGY AND NUTRIENT SENSING PATHWAYS AS REGULATORS OF METABOLIC DISEASE

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BACKGROUND: Metabolic dysfunction underpins a host of chronic cardiometabolic and inflammatory conditions. At the cellular level, AMP-activated protein kinase (AMPK) and mechanistic target of rapamycin complex 1 (mTORC1) are two masters regulators of energy metabolism and cellular homeostasis. The general consensus is that when energy is low, this is sensed by AMPK (via multiple nutrients and signals), which is then activated and acts to conserve cellular energy. When energy and nutrients are plentiful, mTORC1 signals to coordinate anabolic programs. While both AMPK and mTORC1 target dozens of pathways, very little is known about which signals are important in the context of normal and dysfunctional metabolism, as genetic disruption of each kinase leads to a complete collapse of their entire networks. Moreover, while both kinases negatively regulate each other, the physiological significance of these signals is unknown.

HYPOTHESIS: AMPK-mediated inhibition of mTORC1 is critical for homeostasis (this may seem obvious but no one actually knows).

METHODS: We have generated point mutant knock-in (KI) mice where AMPK phosphoregulatory sites thought to control mTORC1 (Raptor S722/S792 and TSC2 S1387) have been mutated to alanine. We have conducted fasting/refeeding experiments, investigated metabolic outcomes during diet-induced obesity, diet-induced fatty liver and a mouse model of atherosclerosis.

RESULTS: Our work has validated that without AMPK regulation, mTORC1 signaling is augmented. Moreover, we show an unexpected and significant role for this regulatory axis in the context of the metabolic switch between fasting and feeding, suggesting an insulin resistance (possibly selective) phenotype whereby there is a dramatic block in insulin-induced lipogenesis/TAG accumulation. This likely explains a maladaptive "protection" from diet-induced obesity and fatty liver. Conversely, higher mTORC1 seems to exacerbate inflammatory and atherogenic readouts.

CONCLUSIONS: This work goes beyond confirming that AMPK restrains mTORC1 in vivo, but points to the importance of endogenous metabolic regulation of mTORC1 for metabolic homeostasis.

P7 LIPOPROTEIN(a) PROMOTES THROMBOSIS THROUGH EFFECTS ON PLATELET AGGREGATION

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Background: Mendelian randomization studies support a causal role for elevated Lipoprotein(a) (Lp(a)) levels in atherosclerotic cardiovascular disease outcomes. However, a mechanistic understanding of this relationship remains elusive. Interestingly, emerging clinical evidence suggests that aspirin use may be beneficial in primary prevention for individuals with elevated Lp(a), suggesting that Lp(a) may possess prothrombotic effects. However, the impact of Lp(a) on thrombosis has not been directly assessed in thrombi formed in vivo.

Hypothesis: We hypothesize that Lp(a) will promote blood clot formation through effects on platelet activation and aggregation.

Methods: Six-week-old transgenic Lp(a) mice (expressing human apo(a) and human apoB-100) and littermate controls (expressing only human apoB-100) were fed a high fat, high cholesterol diet for 6 weeks. In some experiments, mice had their ad libitum water supplemented with low-dose aspirin (25 mg/kg/day). Mice were then subject to either (1) a FeCl3 carotid artery chemical injury model to quantify rates of vessel occlusion using a Transonic Ultrasound blood flow probe, or (2) a tail vein transection injury to assess blood loss volumes using spectrophotometric hemoglobin measurements.

Results: (1) Following FeCl3 carotid artery injuries, rates of occlusive clot formation were significantly increased in both male and female Lp(a) mice compared to littermate controls ($p<0.05$). However, after daily low-dose aspirin treatment, we observed no difference in rates of occlusive clot formation between Lp(a) mice and littermate controls. (2) Following tail vein transections, blood loss volumes were significantly decreased in both male and female Lp(a) mice compared to littermate controls ($p<0.05$). Conversely, preliminary studies suggest that there are no differences in blood loss volumes between Lp(a) mice and littermate controls following low-dose aspirin treatment.

Conclusions: Following in vivo vascular injuries, we show that Lp(a) has prothrombotic properties through apparent effects on platelet activation/aggregation. Taken together, our findings suggest that elevated Lp(a) levels may directly contribute to atherothrombotic events.

INVOLVEMENT OF FIBROBLAST ACTIVATION PROTEIN IN MYOCARDIAL REMODELLING DURING INFARCTION IN A MOUSE MODEL OF CORONARY ARTERY DISEASE

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BACKGROUND: Myocardial infarction, caused by the blockage of blood flow in the coronary arteries, is a major contributor to global morbidity and mortality, often resulting from atherosclerotic processes that lead to coronary artery disease. Following myocardial infarction, myocardial remodeling is a key response that involves complex cellular and molecular mechanisms that contribute to heart failure. During this remodeling, damaged or dead myocardial cells are replaced by fibrous scar tissue produced by activated fibroblasts. While

this process stabilizes the damaged area, it also leads to tissue stiffening, impairing the heart's ability to pump blood and potentially resulting in heart failure. Fibroblast Activation Protein (FAP), a serine protease expressed by activated fibroblasts during tissue remodeling, may play a role in this process, but its specific involvement in myocardial remodeling is not fully understood. In this study, we aim to characterize FAP expression in the heart during myocardial fibrosis.

HYPOTHESIS: We hypothesize that FAP protein levels are associated with myocardial remodeling during infarction in a mouse model of coronary artery disease.

METHODS: We will characterize FAP in the myocardium of SR-B1/ApoE double knockout mice, a model that spontaneously develops occlusive coronary artery atherosclerosis and myocardial infarction, characterized by myocardial fibrosis, elevated plasma markers of cardiac damage and left ventricular dysfunction. Using immunofluorescence staining, we investigated FAP levels in myocardial tissue to elucidate its contribution to remodeling following myocardial infarction.

RESULTS: Our findings suggest a significant association between FAP protein levels and myocardial remodeling, highlighting its potential as a biomarker for and contributor to disease progression.

CONCLUSION: This research contributes to the growing evidence of FAP's role in cardiovascular pathology and may pave the way for future studies on its therapeutic potential in heart disease.

P9 MANNOSE ORAL SUPPLEMENTATION DECREASES ATHEROSCLEROSIS, INFLAMMATORY IMMUNE CELL RECRUITMENT AND IMPROVES MITOCHONDRIAL FUNCTION IN APOE-/- MICE

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Background: Excess dietary sugars are associated with obesity and atherosclerosis. D-mannose, a C-2 epimer of glucose, has been found to regulate immune cell functions and to prevent obesity in high fat diet (HFD) fed mice.

Hypothesis: Mannose alleviates the pro-atherogenic effects of HFD by modulating chronic low-grade inflammation and immune cell metabolism in ApoE-/- mice.

Methods and results: Male ApoE-/- mice were fed a HFD for 9 weeks and concurrently received tap water containing 0, 5, or 20% D-mannose. HFD increased body weight and lipid levels to the same extent in all groups. Atherosclerotic plaque size in the aortic sinus of mice supplemented with 5 or 20% mannose was half of that of 0% mannose mice (20%: 0.11+/-0.02, 5%: 0.17+/-0.03 vs 0%: 0.33+/-0.04mm2). HFD also resulted in a 3-fold increase in blood CD45+/Gr1+ neutrophils compared with animals fed a chow diet; this effect was abolished in mice on HFD + 5% or 20% mannose. Likewise, circulating pro-inflammatory Ly6CHI monocytes were elevated to a lesser extent in HFD mice treated with 5 or 20% vs. 0% mannose. 20% mannose reduced CD45+/Gr1+ neutrophils following HFD. Furthermore, whereas intestinal F4/80+ macrophage numbers were similar, proportions of TLR4-expressing F4/80+ were reduced under 5% (2.0+/-0.4%) or 20% (1.0+/-0.3%) mannose vs. 0% (4.3+/-0.6%). We investigated the metabolic status of bone marrow-derived monocytic cells. Extracellular acidification rate (ECAR) analysis revealed a decrease in glycolysis, glycolytic capacity and reserve in cells from mice receiving 20% mannose vs. 0%. Similarly, mannose was associated with a decrease in the oxygen consumption rate (OCR) marked by reduced basal respiration, ATP-linked respiration, and proton leak.

Conclusions: In ApoE-/- mice, HFD induces inflammatory responses in the gut and the circulation, promoting atherosclerosis. Oral mannose improves the mitochondrial function of monocytic cells, abates intestinal macrophage activation, and reduces blood neutrophil and pro-inflammatory monocytes, resulting in decreased atherosclerosis.

P10 GLUCAGON SIGNALLING IN THE NUCLEUS OF THE SOLITARY IN THE REGULATION OF HEPATIC TRIGLYCERIDE SECRETION IN A MODEL OF TYPE 2 DIABETES

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Background: Glucagon has extensive roles in lipid metabolism via direct actions on peripheral organs, including the liver and adipose tissue and novelly in the brain. Glucagon in the nucleus of the solitary tract (NTS), an important brain region involved in whole-body metabolism, decreases hepatic triglyceride-rich very low-density lipoprotein (VLDL-TG) secretion in healthy rats. This lipid-lowering effect of NTS glucagon is glucagon receptor-dependent and requires the activation of protein kinase A (PKA) within the NTS.

Hypothesis: We aimed to test the hypolipidemic effect of NTS glucagon in diabetic rats, which exhibit elevated VLDL-TG secretion and hypothesized that it lowers hepatic VLDL-TG hypersecretion.

Methods and Results: Male Sprague-Dawley rats underwent stereotaxic NTS bilateral cannulation and vascular catheterizations to enable simultaneous NTS infusions, blood sampling, and intravenous injections. Experimental type 2 diabetes (T2D), induced using combination injections of nicotinamide and streptozotocin and high-fat diet-feeding, was verified with daily monitoring of blood glucose levels. Hepatic VLDL-TG secretion was measured in 10h-fasted rats after intravenous injection of poloxamer, a lipoprotein lipase inhibitor, with concurrent NTS infusions of vehicle, glucagon, or PKA activator (Sp-cAMPS). In T2D rats, NTS glucagon failed to lower VLDL-TG secretion compared to NTS vehicle control. However, direct activation of PKA selectively in the NTS with an infusion of Sp-cAMPS lowered VLDL-TG secretion in hypertriglyceridemic T2D rats. This occurred independent of differences in plasma glucose, insulin, and glucagon, but was associated with lowered plasma levels of free fatty acids.

Conclusions: We provide evidence that although direct glucagon action within the NTS may be impaired in metabolic diseases such as T2D, its downstream signalling targets within the NTS remains intact and is involved in the regulation of lipid metabolism. Understanding the mechanisms of NTS glucagon action may help in the development of therapeutics to treat aberrant blood lipid levels present in metabolic disease states.

P11 ACTIVATION OF VEGFR3 AND MLC2 ARE CRITICAL FOR GLP-2 ENHANCEMENT OF CHYLOMICRON TRANSPORT

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BACKGROUND: A significant proportion of absorbed dietary triacylglycerols (TAG) remain in various intra- and extracellular intestinal compartments for many hours after fat ingestion, including in the lymphatics. TAGs retained in the intestine or lymphatics can be mobilized by the gut peptide, GLP-2, and other stimuli. Our previous published data demonstrated that GLP-2 enhances lymph flow by acting distal to the enterocyte, specifically by enhancing lacteal

contractility, in an enteric nervous system-dependent fashion. The objective of the present study was to further explore various intermediates in the signaling pathway whereby GLP-2 enhances mesenteric lymph flow. In this study we focused on the roles of VEGFR3 (vascular endothelial growth factor receptor 3) and MLC2 (myosin light chain 2), known to play important roles in lymphangiogenesis and lymphatic contractility, respectively.

METHODS: A rat lymph fistula model was utilized in this study. An intraduodenal (i.d.) lipid bolus was administered 5 hours before the following intraperitoneal (i.p.) administrations: 1) saline (placebo), 2) GLP-2, 3) GLP-2 + MAZ-51 (a VEGFR3 inhibitor), 4) GLP-2 + SAR131675 (a second VEGFR3 inhibitor), 5) GLP-2 + ML-7 (a MLCK inhibitor). Lymph flow and TG output were assessed for 60 mins after the i.p. administrations. In another set of animals, post-i.p. administration, tissue samples were collected to quantify VEGFR3 and MLC2 activation (phosphorylation).

RESULTS: GLP-2 treatment significantly increased VEGFR3 (P=0.013) and MLC2 (P=0.009) phosphorylation compared to placebo. Moreover, inhibition of VEGFR3 significantly reduced (P=0.001 & P=0.002) lymph flow and TG output. Partial but significant effects were also observed with MLC2 inhibitor (P=0.019) for lymph flow. Interestingly, MLC2 phosphorylation was significantly reduced (P<0.05) when VEGFR3 phosphorylation was inhibited with MAZ-51/SAR131675.

CONCLUSIONS: These data suggest that GLP-2 enhances intestinal lipid mobilization by significantly increasing the phosphorylation of VEGFR3 and MLC2. Furthermore, VEGFR3 activation is a a crucial upstream regulator of MLC2 phosphorylation in this process.

P12 EARLY ENDOTHELIAL FUNCTION PREVENTS CHRONIC DAMAGE IN DIABETES: UNRAVELLING PROTECTIVE ENDOTHELIAL-SPECIFIC MECHANISMS

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Background: Cardiovascular disease is a significant long-term complication of type 1 and type 2 diabetes. Hyperglycemia could likely induce early damage to the vascular endothelium which may drive chronic cardiovascular events. We are interested in the endothelial release of nitric oxide (NO), a poorly understood mediator of protective endothelial function. We have found that the angiotensin II receptor blocker, telmisartan, has the unique pleiotropic property of acutely and chronically activating NO-dependent endothelial function, independent of its blood pressure-lowering properties. Whether early hyperactivation of NO-dependent endothelial function can improve diabetes-associated cardiovascular outcomes remains unknown.

Hypothesis: Early NO-dependent endothelial function hyperactivation will prevent diabetes-induced vascular damage.

Methods and Results: Ins2+/Akita (Akita) mice, heterozygous for a variant in Ins2, were compared to age/sex-matched Ins2+/+ (control) littermate mice. At 4 weeks post-diabetes onset (blood glucose ≥ 16.6mmol/L), mice were treated with telmisartan (10mg/kg drinking water) or vehicle for eight weeks. At twelve (12) weeks post-diabetes onset, male Akita mice had a 1.4-fold increase in aortic pulse wave velocity (PWV), a marker of arterial stiffness, compared to control male mice. Aorta from male Akita mice had 16.2% greater phenylephrine (PE)-induced smooth muscle constriction and a 20.2% ($p<0.01$) reduction in acetylcholine (Ach)-induced endothelial NO-dependent vasorelaxation. Telmisartan treatment lowered PWV in male Akita mice to control levels. Telmisartan reduced ($p<0.01$) PE contractility in male Akita mice (36.5%) and control mice (66.5%) mice and attenuated the impaired Ach-induced vasodilation, initiating complete rescue of endothelial protective properties. Bulk-RNA seq of endothelial cells with/without telmisartan remains ongoing.

Conclusions: Early interventions with telmisartan prevented arterial stiffness and rescued protective NO-dependent endothelial function, suggesting telmisartan as a potential therapeutic in reducing cardiovascular damage in diabetes. Future directions include investigating Ins2+/Akita /eNOS null mice to prove the causal role between endothelial NO and its protective role in diabetes-induced damage.

P13 CHARACTERIZING INFLAMMATION IN CORONARY ARTERY DISEASE

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Background: Coronary artery disease (CAD) is the build-up of atherosclerotic lesions on coronary arteries, causing reduced blood flow to the cardiac muscles. Recently, numerous clinical trials were launched, aiming to repurpose anti-inflammatory drugs for treating stable coronary artery disease. Most attempts at repurposing were based on clinical observation without knowing what inflammatory targets are present in patients with stable coronary artery disease.

Hypothesis: Specific inflammatory pathways are enriched in human coronary lesions at different pathological stages.

Methods and Results: Multiple coronary arteries from the same patients with heart failure were collected after heart transplants and stored in RNAlater. These patients had stable coronary artery disease by the time of heart transplant. Advanced lesions in these arteries are usually fibrotic or calcified. To digest these "tight" tissues, we incorporated proteinase K into the Qiagen RNeasy Mini Kit protocol. To examine the effect of proteinase K digestion, we cut 5 coronary arteries in half, with one half treated with proteinase K and the other half untreated. Proteinase K led to a higher RNA yield and better detection of the low abundance genes by bulk RNA-sequencing. Moreover, calcification gene SPP1 and mitochondrial genes ND2, ATP6, MTATP6P1 and MTND2P28 were under-represented without proteinase K digestion, suggesting that proteinase K may facilitate the retrieval of RNA sequestered by fibrotic and calcified tissue and the mitochondrial membranes. We performed bulk RNA-sequencing on two pairs of early lesions (pathological intimal thickening) and advanced lesions (late fibroatheroma) from two patients. Gene ontology analysis showed that complement and coagulation cascades were enriched in late fibroatheroma. These suggested a higher inflammation in the advanced lesions.

Conclusions: Proteinase K treatment improved RNA extraction and sequencing quality. The gene expression profiles in advanced lesions showed increased inflammatory processes. Future research could focus on anti-inflammatory therapies that target these pathways.

P14 SHARED REGULATION OF CELLULAR METABOLISM BY PROTEIN TRANSLATION ELONGATION FACTOR 1A (EEF1A) PARALOGS

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BACKGROUND: Elongation factor 1A (EEF1A) exists as two paralogs, EEF1A1 and EEF1A2. Both participate in peptide elongation, but they also have roles in other cellular processes. Using HepG2 cells, which express only EEF1A1, and EEF1A1-deficient CHO-K1

(2E2) cells, which express only EEF1A2, we identified a role for EEF1A1 in maintaining glycolysis at the expense of fatty acid oxidation. However, it is not known whether EEF1A2 shares this function, nor whether the paralogs share a common mechanism of action in regulating cellular metabolism.

HYPOTHESIS: Based on their sequence similarity and shared role in protein translation, EEF1A1 and EEF1A2 have the same function in regulating cellular metabolism.

METHODS AND RESULTS: We measured culture media acidification and lactate accumulation in wild type CHO-K1 and 2E2 cells using two inhibitors of EEF1A. Didemnin B (DB) is non-specific for either paralog, while commercially available plitidepsin (PL) is marketed as an EEF1A2 inhibitor. Protein synthesis measurements in wild type and 2E2 cells treated with either didemnin B or plitidepsin showed similar IC50 values, indicating that both molecules are effective inhibitors of both EEF1A paralogs. Treatment with the IC50 concentrations in wild type (40nM DB and PL) and 2E2 (40nM DB and 20nM PL) cells decreased culture media acidification and lactate accumulation. These data are consistent with decreased glycolysis, suggesting that EEF1A paralogs share a role in maintaining this metabolic pathway. To determine the mechanism underlying EEF1A-mediated metabolic regulation, flux balance analyses will be performed using a metabolic model incorporating multi-omics data from wild type and 2E2 cells.

CONCLUSIONS: Inhibition of either EEF1A1 or EEF1A2 results in decreased glycolysis, suggesting a shared function of these paralogs in maintaining this metabolic pathway. This work has implications for targeting EEF1A in MASLD and cancer, where repression of glycolysis in favour of fatty acid oxidation is a therapeutic goal.

P15 MOBILIZATION OF CHOLESTERYL ESTERS IN SMOOTH MUSCLE CELL FOAM CELLS BY OVEREXPRESSION OF LYSOSOMAL ACID LIPASE

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BACKGROUND: Arterial smooth muscle cells (SMCs) comprise the majority of foam cells in atherosclerotic lesions. Lysosomal acid lipase (LAL), encoded by LIPA, is the sole enzyme mediating cholesteryl ester (CE) hydrolysis to free cholesterol in lysosomes. SMCs express low levels of LAL when compared to macrophages. Despite SMCs also having low expression of ATP-binding cassette transporter A1 (ABCA1), supplementation of SMC foam cells with exogenous LAL decreases lysosomal CE accumulation and increases cholesterol efflux from SMC foam cells.

HYPOTHESIS: Overexpression of LAL reduces lysosomal CE and increases cholesterol efflux from SMC foam cells by ABCA1-dependent and -independent mechanisms.

METHODS: Human SMCs were treated with 0.5 ug/mL LIPA or luciferase mRNA-containing lipid nanoparticles (LNPs) for 3 hours. Cells were loaded with agLDL followed by a 24-hour equilibration period. Cellular LAL activity was measured and lysosomal CE was determined using LAMP-1 (lysosome marker) and BODIPY (neutral lipid) staining. Cholesterol efflux to apolipoprotein A-I (apoA-I) was determined following ABCA1 or control siRNA treatment. Student's t-test was used for statistical analysis of LAL activity, and Mann-Whitney U Test for analysis of colocalization of lipid pools.

RESULTS: LAL activity increased 6-fold 24 h after LNP-LIPA treatment compared to SMCs treated with LNP-Luc or no LNPs ($n=3$, $p < 0.001$). Increased LAL expression in the absence of cholesterol acceptor resulted in a shift in CE storage from lysosomes to non-lysosomal compartments (Pearson correlation coefficient for BODIPY/LAMP-1 colocalization 0.19 in LNP-LIPA vs. 0.34 in LNP-Luc SMCs, $n=87$, $p < 0.0001$). Preliminary cholesterol efflux

studies to apoA-I showed a 3-fold reduction in cellular CE compared to controls and partial inhibition using ABCA1 siRNA.

CONCLUSIONS: LNP-LIPA treatment increases LAL activity in SMCs, reducing lysosomal CE accumulation. Further studies will examine the mechanisms of ABCA1-independent efflux and changes in gene expression following LAL supplementation in SMC foam cells.

P16 CAFFEINE-INSPIRED PCSK9 INHIBITORS FOR THE TREATMENT OF CARDIOVASCULAR DISEASES

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Background Cardiovascular disease (CVD) is among the leading causes of death worldwide. Often exacerbated with sedentary lifestyles and unhealthy diets, abnormal adiposity can increase circulating levels of low-density lipoprotein (LDL) cholesterol, promoting CVD. Current therapeutic strategies consist of lowering 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 potential class of caffeine-inspired therapeutics for such a role.

Hypothesis It has been demonstrated that caffeine blocks liver PCSK9 expression and enhances LDL cholesterol clearance. Although caffeine has been shown to reduce circulating PCSK9 levels, the molecular mechanisms by which caffeine and xanthine-derived compounds lower PCSK9 remains to be fully understood. Thus, the purpose of this study was to further elucidate how caffeine and select caffeine derivatives regulate cholesterol clearance. We postulate that certain synthetic caffeine-derivatives decrease PCSK9 expression/secretion. Methods and

Results The immortalized human hepatocyte cell line HuH7, known to express and secrete PCSK9, were treated with caffeine or our novel caffeine derivatives to assess their ability to maintain/increase endoplasmic reticulum (ER) Ca2+ levels while reducing PCSK9 expression/secretion using ELISAs. Our data confirm and extend previous reports that caffeine increases ER calcium levels in hepatocytes, which leads to reduced PCSK9 expression/secretion. Furthermore, we have synthesized several unique caffeine derivatives with enhanced potency in enhancing ER Ca2+ and lowering PCSK9 versus caffeine. For instance, our three lead derivatives are >1000-fold more potent in increasing ER Ca2+ levels at 6 hours while lowering the secretion of PCSK9 in HuH7 cells after 24 hours.

Conclusions Collectively, these findings have direct clinical implications that could lead to the development of new caffeine-inspired medicines for the treatment and management of CVD.

P17 MONOOXYGENASE X REGULATES COLLAGEN ACCUMULATION IN RESPONSE TO CHEMICAL-INJURY INDUCED LIVER FIBROSIS.

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Background: Metabolic-dysfunction associated steatotic liver disease (MASLD) is a condition characterized by the accumulation of fat in the liver. MASLD can vary from simple steatosis to its most severe form, called metabolic-dysfunction associated steatohepatitis (MASH), which is associated with inflammation and the accumulation of scar tissue (collagen). Liver fibrosis is a

strong independent predictor of mortality in patients with MASH, however, the precise mechanisms that contribute to its progression is not well understood. We found a novel enzyme, Monooxygenase X (MOX), that may be involved in the progression of liver fibrosis. Although MOX currently has no known function, we set out to explore how it contributes to liver disease progression in mice lacking a functional MOX.

Hypothesis: Previous studies have suggested that MOX may be a mediator in liver diseases by showing that its expression correlates with macrophage infiltration and collagen levels, therefore, we hypothesize that mice lacking MOX in the liver may be protected from liver injury.

Methods and Results: We generated hepatocyte-specific knockout of MoxD1 in mice using the Cre-lox recombination strategy. Control mice containing only the MoxD1 floxed gene (MoxD1 Fl/Fl) or hepatocyte-specific knockout mice (MoxD1 Fl/Fl Alb Cre+) were subjected to a biweekly injection of carbon-tetrachloride for a total of 6 weeks. We found that the content of collagen deposition in the liver of mice was reduced in hepatocyte-specific knockout mice compared to control. Although collagen gene expression or markers of hepatic stellate cell activation did not appear to be changed, the expression and activity of MMP-9 was elevated in the livers of hepatocyte-specific knockout mice.

Conclusions: Our findings suggest that hepatocyte-specific MoxD1 may be a contributor to liver fibrosis, which may pose as a potential therapeutic target for MASH patients with liver fibrosis or developing cirrhosis.

P18 VASCULAR SMOOTH MUSCLE CELL SPECIFIC MT1-MMP CAN DECREASE ATHEROSCLEROSIS PROGRESSION IN LDLR KO MICE

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Background Vascular smooth muscle cells (VSMCs) are essential contributors in atherosclerotic plaques development as more than 50% of foam cells within the atherosclerotic plaques are from a VSMC origin; however, to develop a foam cell, VSMCs need to migrate from the medial layer of the artery to the intimal layer. This requires degrading the surrounding extracellular matrix (ECM) components like collagens by proteases. Membrane type-1 matrix metalloproteinase or MMP-14 is expressed in VSMCs in atherosclerosis, it has a role in ECM degradation and promotes VSMC migration/invasion. Here, we investigate the role of MMP1 on atherosclerosis development and VSMC migration/invasion utilizing in-vivo and in-vitro approaches.

Hypothesis VSMC MT1-MMP can increase smooth muscle cell migration, promoting the development/progression of atherosclerotic plaques.

Methods & Results A tamoxifen-inducible VSMC-specific Mmp14 knockout in mice had no significant effect on baseline cardiac function. Primary VSMCs isolated from the aorta of VSMC-specific Mmp14 knockout mice had significantly lower migration/invasion through a collagen type I barrier. Aortic VSMC explant cultured in collagen type I gel was unable to degrade the surrounding collagen compared to the control explant confirming MMP14's role in collagen degradation and VSMC migration/invasion. Atherosclerosis development was investigated in VSMC-specific Mmp14 knockout in adult Ldlr-/- mice. Mice were then fed a high-fat/high-cholesterol diet for 16 weeks to develop atherosclerosis. We found that mice lacking VSMC Mmp14 showed a significant reduction in atherosclerotic plaques with increased collagen content but displayed no significant difference in lipid profile or body weight compared to control mice.

Conclusion VSMC Mmp14 knockout decreased primary VSMC migration/invasion and collagen degradation in-vitro. In-vivo, it didn't affect the baseline characteristics of mice but significantly reduced atherosclerosis progression and increased the collagen content within lesions in Ldlr-/- mice. Understanding the molecular mechanisms of how MM14 modulates these processes and promotes atherosclerosis progression will pave the way for possible therapeutic targets to ameliorate atherosclerosis development.

P19 PDZK1 DEFICIENCY INCREASES DIET INDUCED CORONARY ARTERY ATHEROSCLEROSIS, MYOCARDIAL FIBROSIS AND LEFT VENTRICULAR DYSFUNCTION IN LDLRKO/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 hepatocytes, it plays a significant role in HDL metabolism by binding the carboxy-terminus of the HDL receptor, SR-B1, stabilizing it against degradation. 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. We previously showed PDZK1 inactivation increased aortic sinus atherosclerosis in LDLR KO mice fed an atherogenic diet.

Hypothesis: PDZK1 deficiency increases susceptibility to coronary artery atherosclerosis and myocardial fibrosis due to dysregulated necroptosis.

Methods: $Pdzk1^{KO/KO}Ldlr^{KO/KO}$ and control $Pdzk1^{wt/wt}Ldlr^{KO/KO}$ mice were fed high fat/cholesterol/cholate containing (HFCC) diet for up to 10 weeks. Atherosclerosis in the aortic sinus and coronary arteries (CA) were measured histologically. Cardiac fibrosis was analyzed by trichrome staining. Phosphorylated RIP3K and MLKL were detected by immunofluorescence staining and confocal microscopy. Left ventricular (LV) function was analyzed by invasive hemodynamics using a pressure-volume transducing catheter (PV-Loop analyses).

Results: $Pdzk1^{KO/KO}Ldlr^{KO/KO}$ mice exhibited increased plasma lipoprotein cholesterol levels and atherosclerosis in both aortic sinus and CAs compared to $Pdzk1^{wt/wt}Ldlr^{KO/KO}$ mice. $Pdzk1^{KO/KO}Ldlr^{KO/KO}$ mice had increased levels of cardiac fibrosis, phospho-RIP3K and phospho-MLKL in both aortic sinus and CA atherosclerotic plaques and in the fibrotic regions of the myocardium. LV function was also substantially reduced in $Pdzk1^{KO/KO}Ldlr^{KO/KO}$ compared to $Pdzk1^{wt/wt}Ldlr^{KO/KO}$ mice.

Conclusions: PDZK1 deficiency results in increased HFCC diet-induced aortic sinus and CA atherosclerosis, myocardial fibrosis, and LV dysfunction in $Ldlr^{KO/KO}$ mice, with evidence of increased markers of necroptosis in aortic sinus and CA atherosclerotic plaques, and the remodeling myocardium.

P20 INVESTIGATING MECHANISMS OF ADAMTS13 METALLOPROTEASE DOMAIN LATENCY

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Background: ADAMTS13 is a metalloprotease that degrades large multimers of Von Willebrand Factor (VWF) to attenuate platelet adhesion to damaged endothelium. ADAMTS13 is constitutively active towards its substrate but cannot be inactivated by broad-spectrum inhibitors of metalloproteases. This suggests that ADAMTS13 undergoes substrate-induced allosteric activation in the presence of VWF. The mechanism of this activation is not known. ADAMTS13 circulates in a closed conformation which partially obstructs its capacity to cleave VWF but is not responsible for protection against inhibitors. The hydrophobic S3-S1 specificity pockets in the active site of ADAMTS13 are critical for its interaction with VWF but are not likely to be stable in aqueous environments.

Hypothesis: The hydrophobic active site is responsible for maintaining ADAMTS13 resistance to inhibitors while retaining activity toward VWF. Methods: Full-length ADAMTS13 (FL-ADAMTS13) and truncation variants were incubated with various organic solvents. Changes in proteolytic activity was measured using FRETS-VWF73, and susceptibility to inhibition was determined using alpha-2 macroglobulin (A2M).

Results: The activity of FL-ADAMTS13 increased ~3-fold in the presence of 8% DMSO or 24% ethylene glycol. At higher concentrations, the activity of ADAMTS13 is reduced, likely due to protein denaturation. The activity of truncated ADAMTS13 variants was not increased by these solvents. FL ADAMTS13 incubated with 8% DMSO or 24% ethylene glycol retained their resistance to natural protease inhibitors, unable to cleave the alpha-2 macroglobulin bait region. However, FL-ADAMTS13 incubated with 12% DMSO or 40% ethylene glycol cleaved the alpha-2 macroglobulin bait region, indicating that the active site is accessible to broad-spectrum inhibitors.

Conclusions: Organic solvents can increase the activity of ADAMTS13 when in a closed conformation but not in the open conformation. ADAMTS13 was able to cleave A2M at higher concentrations of organic solvents, suggesting the hydrophobicity of the active site is partially responsible for protecting ADAMTS13 against inhibition.

P21 LP(a) INDUCES A PRO-GLYCOLYTIC AND PRO-INFLAMMATORY RESPONSE IN CORONARY ARTERY AND AORTIC VALVE ENDOTHELIAL CELLS, WITH ALTERED METABOLISM AND REACTIVE OXYGEN SPECIES GENERATION.

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BACKGROUND: High plasma lipoprotein(a) (Lp(a)) is a risk factor for cardiovascular disease including atherosclerosis and calcific aortic valve disease. The mechanisms by which Lp(a) acts remain to be clarified, however. Lp(a) has been shown to elicit a proinflammatory phenotype in endothelial cells, with accompanying changes in metabolism towards glycolytic pathways. To further explore these mechanisms, and to contrast the response of human coronary artery endothelial cells (HCAEC) and human aortic valve endothelial cells (HAVEC), we exposed these cells to purified Lp(a) in vitro and undertook transcriptomic and metabolic analyses.

METHODS: Cultured primary endothelial cells were stimulated with 250 nmol/L of Lp(a) or vehicle control for 2, 4, 8, and 16 hours (HCAEC) or 4, 8, and 16 hours (HAVEC). RNA was isolated, and global gene expression changes were examined by RNA-Seq. qRT-PCR was performed to validate Lp(a) effects on expression of selected genes. Mitochondrial activity was

measured by Seahorse assay, and ROS generation was quantified using commercially available assays.

RESULTS: RNA-seq analysis identified significant changes in HCAEC and HAVEC gene expression after Lp(a) treatment, with 1915 and 444 genes identified as differentially expressed in HCAEC and HAVEC at 16 hours, respectively. Bioinformatic analysis using KEGG pathway showed significant enrichment in metabolic and proinflammatory signaling pathways, with distinct yet overlapping patterns in both HCAEC and HAVEC. Interestingly, the most upregulated gene in both cell types was that encoding the stress response protein heme oxygenase-1 (HMOX-1), which was maximally induced by 50-fold in HCAEC and 7.5-fold in HAVEC. Moreover, Lp(a) increased mitochondrial activity, with a shift towards higher glycolysis, ROS generation, and ER stress in HCAEC.

CONCLUSIONS: The relationship between Lp(a), HMOX-1, ROS and metabolic stress illuminates the mechanisms by which Lp(a) induces endothelial dysfunction in cardiovascular disease, creating pathways for the development of new disease prevention and management strategies.

P22 INTRACELLULAR PLAYERS INVOLVED IN LP(A) BIOSYNTHESIS AND SECRETORY TRAFFICKING

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Introduction: Elevated plasma levels of Lp(a) (lipoprotein(a)) are an independent, causal risk factor for atherosclerotic cardiovascular disease. Plasma Lp(a) concentrations are dictated largely by Lp(a) production rate in hepatocytes rather than catabolic rates. Lp(a) assembles in a two-step process. First, intracellular apo(a) forms a high affinity, non-covalent complex with an LDL-like particle, mediated by apo(a) kringle subtypes 7 and 8 lysine binding sites (LBS7,8). Second, the complex is secreted and apo(a) forms a disulfide bond with apoB, creating the Lp(a) particle. However, the specific intracellular mechanisms of trafficking and lipidation that may present targets for therapeutic disruption, remain unclear.

Methods: The LPA gene encoding apo(a) is not expressed in hepatic immortalized cell lines, thus HuH7 cells were transfected with apo(a) or apo(a) ΔLBS7,8 to model Lp(a) assembly. Lipoprotein secretion was assessed in cells cotransfected with apo(a), and siRNAs directed at VLDL-lipidation proteins. Coimmunoprecipitation was performed to evaluate the interactions between apo(a), apoB and the VLDL ER cargo receptor, SURF4. Confocal and super-resolution radial fluctuation (SRRF) microscopy were used to assess apo(a), apoB and SURF4 intracellular localization.

Results: Western blotting of cells following siRNA transfection showed reduced apo(a) secretion with knockdown of SURF4, TM6SF2, FITM2 and MTP. Coimmunoprecipitation data showed that apo(a) transfection reduced SURF4-apoB binding and was dependent on apo(a)-apoB interaction. Using SRRF microscopy, colocalization of apo(a), apoB and SURF4 was observed.

Conclusions: Our findings suggest a role for several new players in the apo(a)-apoB-100 secretion pathway. We identified SURF4 as an ER-cargo receptor responsible for apo(a)-apoB secretory trafficking through its direct interaction with apoB. Finally, our data show colocalization of apo(a), apoB and SURF4 in secretory vesicles. Next steps will include differentiating the lipidation mechanisms of the LDL-like particle from those of VLDL using metabolic labeling and confirming vesicles containing apo(a), apoB and SURF4 using ground state depletion microscopy.

P23 ARE ANEURYSMS AND OTHER AORTOPATHIES DISEASES OF THE ENDOTHELIUM? INTRODUCING THE NITRIC OXIDE (NO)-DEPENDENT THERAPEUTIC ENDOTHELIAL FUNCTION RESERVE.

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BACKGROUND: Aortopathies predispose to dilation, aneurysm, dissection, or rupture of the aorta. They can be acquired (atherosclerosis, aging) or genetic (such as Marfan syndrome; MFS), and they are typically managed with blood pressure (BP)-lowering medications although the later lead to underwhelming improvements in outcomes. We have observed heterogenous increases in endothelial nitric oxide (NO) synthase (eNOS)-derived NO release, a protective mediator of endothelial function and vascular homeostasis, with certain aortopathy-compatible BP medications.

HYPOTHESIS: Endothelial NO has greater anti-aortic remodeling effects than BP lowering, which if optimized will lead to grater

METHODS AND RESULTS: Dose-response experiments revealed that telmisartan, a lipophilic angiotensin II (AngII) receptor blocker (ARB), is the most effective activator of NO release, leading to a 91% inhibition of aortic contractility in a fully NO-dependent fashion when use at high doses. Telmisartan completely (100%) inhibits aortic widening in age- and MFS-associated models of aortopathy, which far exceeds clinical outcomes with other, more established BP medications. Interestingly, low, sub-BP doses of Telmisartan can also activate vascular NO release and inhibit by up to 72% aortopathy progression. Inhibition of eNOS completely (100%) blocks telmisartan's anti-aortic remodeling properties without interfering with its BP-lowering effects.

CONCLUSION: Our results suggest that most of the therapeutically-relevant properties of the endothelium may be dormant – the therapeutic endothelial function reserve (tEFR) – and that proper stimulation with certain BP medications lead to unexpected release of NO through the tEFR. The causal role of tEFR vs BP lowering in therapeutic responses in aortopathies and other diseases such as atherosclerosis is currently under investigation.

P24 THE ADMINISTRATION OF APOA1 SELECTIVELY PROTECTS CARDIOMYOCYTES WHILE MAINTAINING THE CHEMOTHERAPEUTIC EFFICACY OF DOX IN THE SAME PRE-CLINICAL MODEL.

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BACKGROUND*: Doxorubicin* (DOX) is a commonly used and effective chemotherapeutic agent. It has been the backbone of breast cancer therapy for decades. However, DOX causes cardiotoxicity which can lead to life-threatening long term effects on cardiac function. High-density lipoprotein (HDL) levels are inversely correlated with risk for heart disease and pre-clinical studies have demonstrated that HDL can directly protect hearts against the effects of ischemic disease. HDL has also served as the inspiration for the design of synthetic nanoparticles to encapsulate DOX and these have been shown to be highly effective at delivering DOX to tumors. HDL is generated in vivo when Apolipoprotein (Apo) A1, secreted into the blood, acquires phospholipids and cholesterol from cells. We have previously reported that overexpression of a human Apolipoprotein (Apo) A1 transgene or injection of human Apo)A1 protein into mice protects them against DOX-induced cardiotoxicity.

HYPOTHESIS ApoA1 treatment can selectively protect against cardiotoxicity while maintaining the chemotherapeutic efficacy of DOX in the same pre-clinical model.

METHODS AND RESULTS We generated a breast tumor graft model by injecting murine 4T1 breast tumor subcutaneously into the mammary gland of female Balb/c mice. One week after inoculation with tumor cells, mice were treated intraperitoneally with weekly injections of either DOX alone, or DOX along with ApoA1. Control mice received weekly treatments with ApoA1 alone or with saline. Tumor sizes were measured weekly with calipers After 4 weeks of treatment with DOX alone, DOX + ApoA1, ApoA1 alone or saline, mice were euthanized and tumors were excised, and measured. Hearts were collected and cardiotoxicity was analyzed by measuring cardiomyocyte cross sectional area and apoptosis in histological sections. ApoA1 treatment did not affect DOX mediated reduction in tumor growth, but it did reduce the levels of DOX induced apoptosis in hearts as well as DOX induced reduction in cardiomyocyte cell cross sectional area.

CONCLUSIONS These results demonstrated that ApoA1 injection selectively protects against DOX induced cardiotoxicity by inhibiting both DOX induced cardiomyocyte apoptosis and atrophy; whereas it does not impact DOX's ability to attenuate tumor growth. This suggests ApoA1 may have potential as a complementary therapy to reduce DOX-induced cardiotoxicity without affecting its antitumor efficacy.