KEENAN RESEARCH CENTRE FOR BIOMEDICAL SCIENCE

LI KA SHING KNOWLEDGE INSTITUTE

ST. MICHAEL'S HOSPITAL

2019

Trainee Research Day

RESEARCH TRAINING CENTRE

NOVEMBER 4, 2019 - 9AM-6PM

St. Michael's

Inspired Care. Inspiring Science. | Research Training Centre

About the Research Training Centre (RTC)

The Research Training Centre (RTC) is part of the larger Research Institute at St. Michael's Hospital, which is comprised of the Keenan Research Centre for Basic Science (KRCBS) and the Li Ka Shing Knowledge Institute (LKSKI). The RTC seeks to provide a stimulating research training program and to create a nationally and internationally recognized training environment for future scientists. By bringing together expertise in translational scientific and applied health services research, the RTC works with trainees and the broader research community to support and advance their research training experiences.

What kind of trainees is the RTC supporting?

We provide support for individuals conducting research at St Michael's Hospital as a graduate student (at either the Master's or Doctoral level) or as a Postdoctoral Fellow, and under the supervision of a St. Michael's Hospital scientist; as well we also provide support to supervisors who participate in research training.

A graduate student is an individual enrolled in a graduate program pursuing a Masters or PhD degree which is focused on research.

A postdoctoral fellow (PDF) is an individual with a PhD degree who is pursuing post-doctoral research training.

Contact Us

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http://stmichaelshospitalresearch.ca/research-training-centre/

TRAINEE RESEARCH DAY AGENDA

Date: **Monday, November 4th, 2019**
Time: **9:15am - 6:00pm**
Location: **The Allan Waters Family Auditorium / Exhibition Hall**

Registration and Breakfast **8:30am to 9:15am**
Students and honoured guests will sign in. Poster presenters to set up in Bernie and Mildred Syron Exhibition Hall. Tea, coffee and a light breakfast will be available.

Executive Welcome **9:20am to 9:25am**
Dr. Janet Parsons & Dr. Katalin Szaszi, Co-Directors of the RTC

First keynote: Dr. John Marshall **9:30am to 10:15am**
Speaking Science

Break **10:15am to 10:30am**

Oral Presentations – Experimental/ Basic Science Research **10:30am to 12:00pm**

Lunch **12:00pm to 12:45pm**

Oral Presentations –Clinical/ Health Policy Research **12:45pm to 2:15pm**

Poster Competition: Open Viewing **12:00pm to 6:00pm**
Posters will be open for viewing.

Poster Judging **2:30pm to 3:30pm**
Judging committee will evaluate posters. Presenters are given five minutes to present and five minutes for questions.

Second keynote: Dr. Muhammad Mamdani , Li Ka Shing Knowledge Institute **3:45pm to 4:30 pm**
Six Things Every Research Trainee Should Know About Healthcare Research

Award Ceremony **4:30pm to 4:45 pm**
Awards ceremony in the Bernie and Mildred Syron Exhibition Hall / The Tony and Anne Arrell Classrooms.

Networking **4:15pm to 5:00pm**

Wine and cheese networking event in the Bernie and Mildred Syron Exhibition Hall / The Tony and Anne Arrell Classrooms.

2019 Keynote Presenters

"Speaking Science" – 9:30am to 10:15am

John Marshall, MD, FRCSC, FACS, FCAHS

Co-Director, Critical Illness and Injury Research Centre

Scientist, Keenan Research Centre for Biomedical Science

Trauma Surgeon and Intensivist, St. Michael's Hospital

Professor, Department of Surgery, University of Toronto

2018 Keenan Legacy Award Winner – St. Michael's Hospital

After a brief stint as a filmmaker and an aspiring successor to Ingmar Bergman, François Truffaut, and Michelangelo Antonioni, Dr. Marshall obtained his medical degree from the University of Toronto in 1977, and changed the medium of his editing passion from celluloid to flesh. He completed a fellowship in General Surgery at Dalhousie University, Halifax, in 1984, and undertook a research fellowship at McGill University under the mentorship of Dr. Jonathan Meakins. Following 3 years of a critical care surgery practice in Halifax, culminating in the receipt of the Royal College Medal for Surgery in 1989, he moved to Toronto in 1990. He spent 15 years at the Toronto General Hospital as a critical care surgeon and intensivists before moving to St. Michael's Hospital in 2005, where, in addition to the above, he reawakened a long-standing interest in trauma.

Dr. Marshall is currently a Professor of Surgery at the University of Toronto. He runs a CIHR-funded laboratory at St. Michael's Hospital, focused on the mechanisms of prolonged neutrophil survival in sepsis. In addition, he serves as chair of the Canadian Critical Care Trials Group – the oldest and most productive investigator-led critical care clinical trials group in the world. He is a past-president of the Surgical Infection Society, and past-chair of the International Sepsis Forum. Dr. Marshall has published more than 210 papers and 75 chapters, and given more than 600 invited lectures around the world.

"Six Things Every Research Trainee Should Know About Healthcare Research" – 3:45pm to 4:30pm

Muhammad Mamdani, MPH, MA, PharmD

Director, Li Ka Shing Centre for Healthcare Analytics Research and Training (CHART)

Scientist, Li Ka Shing Knowledge Institute, St. Michael's Hospital

Professor, Institute of Health Policy, Management, and Evaluation, University of Toronto

Professor, Leslie Dan Faculty of Pharmacy, University of Toronto

Adjunct Professor, King Saud University Senior Adjunct

Scientist, Institute for Clinical Evaluative Sciences

Dr. Mamdani is the Director of the Li Ka Shing Centre for Healthcare Analytics Research and Training (CHART) of the Li Ka Shing Knowledge Institute of St. Michael's Hospital in Toronto. He is also Professor in the Leslie Dan Faculty of Pharmacy, the Department of Medicine of the Faculty of Medicine, and the Institute of Health Policy, Management and Evaluation of the Dalla Lana Faculty of Public Health. He is also adjunct Senior Scientist at the Institute for Clinical Evaluative Sciences (ICES). Dr. Mamdani also is a member of the Human Drug Advisory Panel of the Patented Medicine Prices Review Board (PMPRB) and is a co-Principal Investigator of the Ontario Drug Policy Research Network. In 2010, Dr. Mamdani was named among Canada's Top 40 under 40. Prior to joining the Li Ka Shing Knowledge Institute and St. Michael's Hospital, Dr. Mamdani was a Director of Outcomes Research at Pfizer Global Pharmaceuticals in New York. Dr. Mamdani's research interests include pharmacoepidemiology, pharmacoeconomics, and drug policy. He has published approximately 350 research studies in peer-reviewed medical journals, including leading journals such as the New England Journal of Medicine, the Lancet, the Journal of the American Medical Association, the British Medical Journal, and Annals of Internal Medicine.

Dr. Mamdani obtained a Doctor of Pharmacy degree (PharmD) from the University of Michigan (Ann Arbor) in 1995 and subsequently completed a fellowship in pharmacoeconomics and outcomes research at the Detroit Medical Center in 1997. During his fellowship, Dr. Mamdani obtained a Master of Arts degree in Economics from Wayne State University in Detroit, Michigan. He then completed a Master of Public Health degree from Harvard University in 1998 with a concentration in quantitative methods, focusing on biostatistics and epidemiological principles.

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Oral Presentations

AMIN EKTESABI: MIR-187 REGULATION IN PRIMARY CARDIOMYOCYTE AND MURINE MODEL OF EXPERIMENTAL SEPSIS-INDUCED MYOCARDIAL DYSFUNCTION

Supervisor: **Dr. Claudia dos Santos**

Background: Multiple microRNAs (miRs) are dysregulated during myocardial sepsis. Systemic administration of mesenchymal stromal/stem cells (MSCs) mitigates sepsis induced myocardial dysfunction and alters the expression of both miRs and their target mRNAs in the septic heart. In an experimental model, we have identified miR-187 as a putative host-derived MSC-regulated miR. Here we investigate, in vitro and in vivo, the in-silico hypothesis that miR-187 plays a critical role in the pathogenesis and therapeutics of sepsis-induced myocardial dysfunction.

Methods: Male wild-type (C57Bl/6J, 10-14 weeks) were randomized to sham or cecum ligation and puncture (CLP) and further randomized to MSCs (2 x 105 cells, tail vein) or placebo, 6 hours post surgery. Mice were sacrificed at 28hrs and hearts collected for protein, histology and RNA analysis. Transthoracic echocardiograms were performed at 48 hrs in a separate group of mice. Primary cardiomyocytes were harvested from 1-2 days old neonates and exposed to endotoxin (lipopolysaccharide, 2µg/mL) or IL-10 (10 ng/ml) ± MSCs (1x104 cells/well). Cells were lysed, RNA isolated 24 hours post-treatment, and analyzed using qRT-PCR.

Results: MSC administration mitigated CLP-induced left ventricular dilatation and decreased ejection fraction. Quantitative real-time PCR confirmed differential expression of pre-identified in-silico targets in-vivo and IL-10, an anti-inflammatory cytokine, in murine septic hearts treated with MSCs. In vitro, miR-187 expression levels were significantly lower in primary neonatal cardiomyocytes, exposed to endotoxin while the expressions of its putative target genes was increased. Similarly, IL-10 expression was decreased in LPS treated cells; this was mitigated by MSC administration.

Conclusion: MSC administration results in the regulation of host-derived miRNAs involved in protecting cardiomyocytes from sepsis-induced inflammation.

Supported by the Canadian Institutes of Health Research (Grant # MOP-130331 to CCDS), the Canada Research Chair in Infectious Diseases and Inflammation to WCL, and the Ontario Research Fund (Grant # RE07-086 to DJS, SHJM and CCDS)

DANIEL MACKEIGAN:
APOLIPOPROTEIN A-IV IS AN ENDOGENOUS INHIBITOR OF THROMBOSIS: THE ROLES OF POLYMORPHISMS IN THE RISK OF CARDIOVASCULAR DISEASE

Supervisor: **Dr. Heyu Ni**

Thrombosis, a common cause of cardiovascular disease, occurs when dysregulated platelets adhere and aggregate in vasculature causing vessel obstruction. We recently identified apolipoprotein A-IV (apoA-IV) as an endogenous inhibitor of the platelet αIIbβ3 integrin which plays a critical role in platelet adhesion and aggregation. Common human polymorphisms in the C-terminal of this protein, Q360H and T347S, are associated with increased incidence of cardiovascular disease. However, the mechanism of action of these polymorphisms on platelet function has never been previously explored. We hypothesize that C-terminal polymorphisms in apoA-IV alters its structure to mask the N-terminal binding site, thus weakening its affinity for the platelet αIIbβ3 integrin and decrease its anti-thrombotic effect. We used site-directed mutagenesis to generate both Q360H and T347S recombinant apoA-IV polymorphisms. Using light transmittance aggregometry, we demonstrated that wildtype apoA-IV exhibits a stronger reduction in ADP and collagen induced platelet aggregation as compared to both apoA-IV polymorphisms, with the T347S mutation showing half the inhibition, and the Q360H mutation showing almost no inhibition. These results were further corroborated using human gel-filtered platelets. Taken together, these results indicate that apoA-IV polymorphisms exhibit reduced inhibition of platelet aggregation. Through ex vivo perfusion chamber microscopy, we revealed that consistently with our previous findings, wildtype apoA-IV protein decreased platelet adhesion to collagen and subsequent aggregation under low shear conditions. However, the T347S mutation resulted in increased platelet adhesion and aggregation while the Q360H mutation abrogated any decrease in platelet adhesion and aggregation. In conclusion, our data reveals a novel mechanism of action of the apoA-IV Q360H and T347S polymorphisms though the decreased blockade of the platelet αIIbβ3 integrin, thus increasing the incidence of cardiovascular disease.

Elyse Latreille: Identification of a novel compound that reduces mortality from severe influenza

Supervisor: **Dr. Warren Lee**

Despite annual vaccination programs, the influenza virus causes hundreds of thousands of deaths annually. There is also a constant threat of a novel strain developing, leading to another pandemic. Currently, three classes of antiviral drugs exist for the treatment of infected individuals. However, due to rapid development of antiviral-resistant strains of influenza, one class is no longer clinically useful. Furthermore, the efficiency of the dominant class of antivirals is controversial, and sporadic cases of resistance have been reported. As such, novel therapeutics for the treatment of influenza are urgently needed. Using a zebrafish model of lethal influenza virus infection, we screened multiple compound libraries to identify compounds that reduced edema and prolonged survival of infected zebrafish. One of the identified compounds was tested in a mouse model, where it rescued mice from lethal influenza infection, even when administered beginning 24 hours after infection. In vitro and in vivo experiments revealed that the compound does not affect viral titer, suggesting that it does not act directly on the virus. Thus, we hypothesized that this drug exerts its protective effects by acting on the host, given that an excessive host immune response is known to be associated with poor outcomes during influenza infections. We found that incubation of influenza-infected lung endothelial cells with the compound greatly attenuated the influenza-induced expression of Intercellular adhesion molecule 1 at both the protein and mRNA level. Additionally, the compound prevented the production of reactive nitrogen intermediates by endotoxin-stimulated murine macrophages by reducing the expression of inducible nitric oxide synthase, without affecting Toll-like receptor 4 expression. Taken together, these results suggest that this compound exerts its protective effect by preventing the excessive and damaging recruitment and activation of leukocytes to the infected lung and could potentially be used a treatment for influenza.

MIHAILS DITMANS: OPTIMIZING DRUG DELIVERY IN ULTRASOUND AND MICROBUBBLE TREATMENT OF ARDS

Supervisor: **Dr. Warren Lee & Dr. Michael Kolios**

Acute Respiratory Distress syndrome (ARDS) is characterized by heterogeneous pulmonary edema, with areas in the same patient varying from relatively healthy and air-filled to severely injured and fluid-filled. This heterogeneity makes ARDS difficult to treat, as inhaled treatments preferably push air into the healthy regions where drugs are unnecessary and the ventilated air can cause lung damage by over-distention. Oral and injected drugs can also cause off target effects, calling for a targeted treatment for ARDS. Our lab has shown that we can deliver therapeutic cargoes to the most injured areas of the lungs using Ultrasound-mediated Microbubble delivery (USMB). The ultrasound waves are reflected by air in healthy lung portions and are propagated by the fluid filled injured portions, allowing the energy to cavitate intravenously injected microbubbles loaded with cargo, stimulating cargo delivery. We have shown USMB-mediated enhanced transfection of Tie2, which is diminished in injured lung tissue, as well as uptake of Evan's blue dye, to the injured lung in a porcine model. We aim to enhance delivery of therapeutic plasmid cargo by altering microbubble cavitation through ultrasound setting manipulation, as well as explore the biological implications of delivering Tie2 plasmid. For this purpose, we have adapted a murine model of unilateral lung infection, which will be performed with either LPS-induced injury for plasmid delivery or flu-induced injury to study the biological effect of USMB-mediated Tie2 transfection. Preliminary infection studies show decreased Tie2 levels in the injured lung compared to the healthy internal control, serving as a baseline of Tie2 level in the animal. We have also developed a cell model to apply USMB to a confluent endothelial cell monolayer, allowing testing of multiple variations of ultrasound settings and their effects on plasmid delivery. This project will contribute to the future development of an effective targeted treatment for ARDS.

RAMSHA KHAN: IN VITRO PLATELET PHAGOCYTOSIS: A POWERFUL TOOL FOR PREDICTING THE THERAPEUTIC POTENTIAL OF ERYTHROCYTE ANTIBODIES IN MURINE ITP

Supervisor: **Dr. Alan Lazarus**

Ramsha Khan, Melissa Menard, Chao-Ching Jen, Xi Chen, Peter Norris and Alan H. Lazarus

Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder characterized by low platelet counts. Polyclonal anti-D, an erythrocyte-specific plasma derived antibody, is a first line therapy for ITP. However as a donor derived antibody, anti-D is associated with certain limitations and as such, there is interest present in developing a recombinant replacement. Monoclonal antibodies are desirable alternatives to anti-D but none have yet proven clinically successful. Here we examined twelve murine erythrocyte-specific antibodies of different specificity, isotypes and glycosylation states to determine features characteristics of therapeutically successful antibodies. All antibodies were evaluated for their ability to sensitize erythrocytes, ameliorate ITP, induce anemia, cause erythrocyte phagocytosis as well as prevent platelet phagocytosis. In vivo, eight antibodies were found to induce significant anemia in antigen positive mice while only five of these ameliorated ITP. Subsequent in vitro examination via phagocytosis assays demonstrated that only the antibodies which supported erythrocyte phagocytosis in vitro also inhibited platelet phagocytosis in vitro and successfully ameliorated ITP in vivo. We conclude that an antibody which can induce anemia is not a sufficient condition for amelioration of ITP but it is the antibody's ability to prevent platelet phagocytosis in vitro that is related to its ability to ameliorate ITP. We suggest that in vitro platelet phagocytosis may prove to be a valuable tool for testing the viability of erythrocyte specific antibodies for clinical use in ITP.

SHENHAB ZAIG: UNDERSTANDING OPIOID-INDUCED RESPIRATORY DEPRESSION AND ANALGESIA USING NOVEL ZEBRAFISH MODELS

Supervisor: **Dr. Gaspard Montandon**

Introduction: Opioids are the gold standard drug for pain management but cause respiratory depression, leading to fatal overdose. It is therefore critical to find safe opioid pain therapies that produce analgesia without respiratory depression. Zebrafish models, which reproduce quickly, are ideal for drug screening research. They share a high homology in their μ-opioid receptor (MOR) system and neural respiratory network to mammals. Therefore, we aimed to develop zebrafish models of opioid-induced respiratory depression and analgesia, to determine their suitability for high-throughput screening of safe opioid pain therapies.

Methods: To determine pain and analgesia, we quantified swimming velocity to 0.05% formalin (nociceptive stimulus) and fentanyl (opioid) in 12-14-day post-fertilization (dpf) larvae. We measured mandible movements as an index of respiratory activity in response to opioids and respiratory stimulants. Splice morpholinos were used to knockdown MORs and the swimming response to fentanyl was measured.

Results: All values were normalized to an individual's baseline activity (baseline=100%). Formalin significantly increased swimming velocity by 260% relative to controls ($p<0.001$). 3μM fentanyl with formalin produced no difference in velocity. 1μM fentanyl significantly decreased breathing rate by 52% compared to controls ($p=0.005$). Naloxone (5μM) and CTAP (4μM), opioid receptor antagonists, blocked the fentanyl's effect ($p=0.002$). Respiratory stimulants significantly increased respiratory rate after fentanyl administration ($p=0.024$). Preliminary data shows that MOR knockdowns increase swimming velocity in response to fentanyl compared to controls.

Conclusion: Fentanyl blocks pain in zebrafish and induces respiratory depression, which can be blocked by naloxone and CTAP. Respiratory stimulants increase respiratory rate after opioid administration. The differential response to opioids in MOR knockdowns suggests a role of the MOR system in mediating these effects. Overall, our data shows that zebrafish respond to opioids similarly to mammals, suggesting that they are a suitable model for developing a high-throughput screening platform to identify safe opioid pain therapies.

AMALIA GIL: INTEGRATION OF AN EYE-TRACKER IN THE OPERATING ROOM TO ASSESS THE EFFECT OF DISTRACTIONS ON SURGEONS' VISUAL ATTENTION

Supervisor: **Dr. Teodor Grantcharov**

Introduction: Over 20,000 preventable patient deaths occur annually in Canada and over half are attributed to surgical procedures. A suspected major contributing factor is the high number of distractions in the operating room (OR). Distractions are any disturbances that may divert attention away from critical surgical tasks such as loud noises, phones ringing, pagers, and doors opening. It remains unclear which distractions affect the surgeons' attention versus those that are present but have no effect. This information can aid in the development of targeted mitigation strategies to reduce the prevalence and effect of distractions to improve patient safety.

Methods: An eye-tracker will be used to assess surgeons' visual attention. The gaze metrics used to measure visual attention are gaze-points, fixation frequency on areas-of-interest (AOI), dwell time on AOI, blink rate, and pupil rate of change. We will then assess temporal correlations between visual attention and distractions, which will be coded using a comprehensive operative capture platform (OR Black Box®). The study will also assess if different distractions have a differential effect on visual attention and if this effect differs according to the surgeon's experience level.

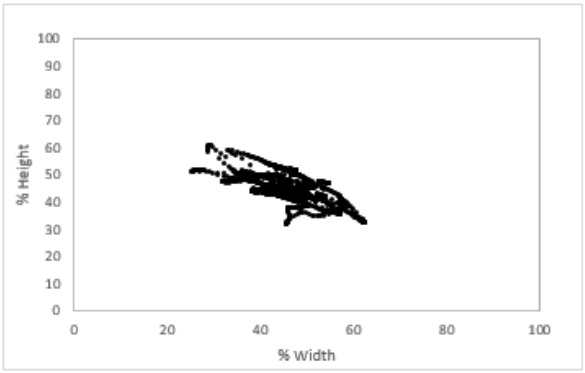
Results: A custom monitor-mounted eye-tracker was developed in collaboration with an industry partner (EyeTech Digital Systems, Inc.) and the required mechanical and electrical installations in the OR have been completed (Figure 1-A-B). The eye-tracker has also been pilot tested in simulation and bench-top environments to understand data outputs and to mitigate risks prior to OR trials; with qualitative differences seen between distracted and non-distracted states (Figure 1-C-D).

Conclusions: An eye-tracker can effectively be integrated into the OR and it will be used to assess how distractions affect surgeons' visual attention.

A - Eye-Tracker

B - Eye-Tracker Testing in Operating Room

C – Gaze on Monitor no Distractions



D – Gaze on Monitor with Distractions

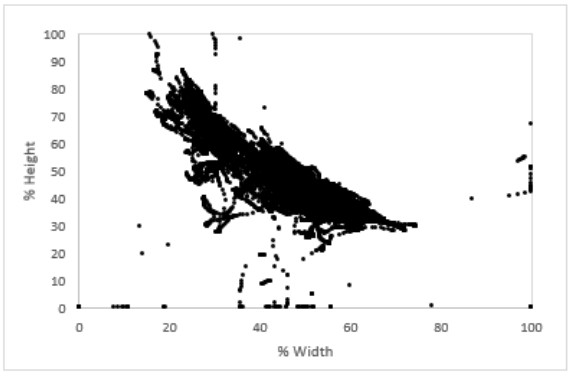


Figure 1. Eye-tracker integration into the operating room and results from pilot simulation tests.

A custom eye-tracker was developed (A) and all required electrical and mechanical installations were completed to install the eye-tracker in the operating room (B). The eye-tracker was also pilot tested in simulation. Graphs C and D present the results of the participant's gaze-points on the surgical monitor with and without distractions. Graph D demonstrates that with distractions the gaze-points had a greater dispersion compared to the gaze-points without distractions.

ANDREA GLENN: LONGITUDINAL CHANGES IN ADHERENCE TO THE DASH AND PORTFOLIO DIETARY PATTERNS AND CARDIOMETABOLIC RISK FACTORS IN PREDIMED-PLUS

Supervisor: **Dr. John Sievenpiper**

Introduction: The Dietary Approaches to Stop Hypertension (DASH) and Portfolio diets have been shown to lower cardiometabolic risk factors in randomized controlled trials (RCTs). To date, the Portfolio diet has only been assessed in RCTs of hyperlipidemic patients and its association with cardiometabolic risk factors in other populations has not been assessed.

Methods: Longitudinal analysis of one-year data from PREDIMED-Plus (6,636 elderly participants with overweight/obesity and metabolic syndrome). Data collection occurred at baseline, 6 months and 1 year. Adherence to the DASH and Portfolio diets were derived from the validated 143-item food frequency questionnaires. Linear mixed models were used to estimate longitudinal associations between diet indices (as continuous and quartile variables) and cardiometabolic risk factors. Models were adjusted for potential confounders.

Results: Greater adherence to the DASH diet was significantly associated with lower HbA1c (β [95% CI]: -0.003 [-0.006, -0.001]), glucose (-0.25 [-0.34, -0.15]), triglycerides (-0.69 [-0.89, -0.48]), non-HDL-C (-0.15 [-0.27, -0.03]), waist circumference (WC) (-0.09 [-0.12, -0.06]), BMI (-0.03 [-0.04, -0.02]) and higher HDL-C (0.04 [0.02, 0.07]) over 1 year of follow-up. Greater adherence to the Portfolio diet was significantly associated with lower HbA1c (-0.004 [-0.006, -0.002]), glucose (-0.11 [-0.19, -0.03]), WC (-0.09 [-0.12, -0.05]) and BMI (-0.03 [-0.04, -0.03]) over 1 year of follow-up. Similar associations were found when diet scores were assessed as quartiles, and the DASH score was further significantly associated with lower diastolic blood pressure in quartile 4 (Q4) compared to Q1.

Conclusion: Among elderly adults at high CVD risk, greater adherence to both diets showed significant favourable associations with many cardiometabolic risk factors, with the DASH diet showing more favourable relationships. Both dietary patterns may therefore be beneficial for CVD and diabetes risk reduction.

Trial registration: ISRCTN89898

CHRISTINE SCHEMITSCH: THE ASSOCIATION BETWEEN PSYCHOLOGICAL FACTORS AND OUTCOME FOLLOWING TREATMENT FOR ROTATOR CUFF DISEASE – A SYSTEMATIC REVIEW

Supervisor: **Dr. Aaron Nauth**

Introduction: Rotator cuff injuries represent a significant burden to the health care system, affecting more than 30% of the population over the age of sixty. The main purpose of this study was to conduct a systematic review to determine the impact of psychosocial factors on the outcome of treatment in patients with rotator cuff disease.

Methods: A systematic search was conducted of Medline, CINAHL, and PsychInfo databases. Titles and abstracts were screened for all studies obtained from the initial search and a full text review was conducted on those studies meeting the eligibility criteria.

Results: 1252 studies were identified, of which 46 underwent a full-text review. Ten studies were included in the final analysis (Table 1).

Three studies examined patient expectations prior to treatment and found that higher expectations led to a significantly improved outcome following both operative and non-operative treatment.

Two studies demonstrated that patients with worse pre-operative general psychological scores had increased post-operative pain.

Four studies assessed the impact of pre-operative anxiety and depression on outcomes following surgical treatment of rotator cuff disease. Only two of the studies found that pre-operative anxiety and depression negatively affected post-operative pain and function.

Finally, one study examined the impact of general distress on outcomes following the surgical treatment of rotator cuff disease and found no association with post-operative levels of pain or function.

Conclusion: The results of this systematic review indicate that there is somewhat conflicting and contradictory evidence within the literature. Overall, however, there does appear to be a positive association between pre-operative psychological factors

and post-operative function and pain. The majority of these studies, however, have had insufficient sample sizes and are often underpowered. To address these limitations, we have designed a large, multi-centre, prospective, observational cohort to clarify the impact of psychological factors on post-operative rotator cuff outcomes.

Table 1. Patient Characteristics of the ten included studies

Total sample size	**1358 (58 – 433)**
# of patients with a complete f/u	**1206 (44 – 407)**
Mean follow-up rate	**89%**
Average follow-up, years	**1 (0.5 – 2.5)**
Mean Age	**58 years**
Sex, % females	**44%**

FARAH N. MAWANI: BUILDING ROADS TOGETHER: A COMMUNITY-BASED WALKING AND ROLLING PEER SUPPORT PROGRAM FOR INCLUSION AND MENTAL HEALTH

Supervisor: **Dr. Patricia O'Campo**

Setting (Introduction): The program founder selected Regent Park for Building Roads Together (BRT) © pilot program implementation because it is one of 31 neighbourhoods identified by the City of Toronto as a Neighbourhood Improvement Area (NIA) based on a low Neighbourhood Equity Benchmark score indicating that it faces serious inequities requiring immediate action; has a higher than average proportion of residents who are recent immigrants; and is Canada's first social housing development undergoing a 25 year process of transformation to a mixed-income community. Community partners confirmed that BRT responded to community needs and complemented existing programs and supports.

Intervention (Methodology): BRT is an award-winning community-based peer support walking and rolling program designed to promote inclusion and reduce health inequities. Strong bodies of evidence demonstrating that peer support; walking; and exposure to green space, alone and in combination reduce social isolation, and improve health, and mental health. The program founder designed BRT based on this research evidence, and a needs assessment including interviews, focus groups, and meetings.

Outcomes (Results): The needs assessment informed program design, including name, goals, approach, content, and curriculum. BRT includes the following phases: 1. Community engagement; 2. Partnership development; 3. Neighbourhood-based Walk the Talk Advisory Groups; 4. Peer Walking/Rolling Group Leadership Training; 5. Mentoring/Support; 6. Peer Walking/Rolling Groups. The training curriculum combines peer leadership, inclusion, and communication skills; practical skills required to create and manage a walking group; and information about urban green space.

Implications (Conclusions): In partnership with the Centre for Learning & Development Toronto (CCLD), and Regent Park Community Health Centre (CHC), the program founder trained 42 peer walking group leaders, and mentored multiple walking groups.

Table 1: Building Roads Together Design Features and Program Content

DESIGN FEATURES	PROGRAM CONTENT
PROGRAM NAME	
Building Roads Together	
PROGRAM GOAL	
To promote inclusion and reduce health and mental health inequities by building capacity for people to lead peer walking groups in urban green space.	
PEER SUPPORT	
Start first training program session by asking program participants "What does peer support mean to you?" and share meanings from needs assessment.	• people with lived experience of mental health issues • people with lived experience of exclusion • people who share common experience; have walked similar paths • Understanding: "Yeah that happens to me too." • Helping each other, supporting each other
Apply and share Building Roads Together working definition of peer support based on synthesis of needs assessment findings	*Peer support is support given and received by people with lived experience of exclusion (associated with social determinants) and/or mental health issues, who have walked similar paths (due to migration, health issues, etc.).*
Integrate peer support principles into program design and implementation, including community engagement, outreach, training, and walking/rolling groups	• Peer leadership • Based on shared experience • Shared leadership: Collegial, collaborative, no hierarchy: no leader and follower, people on same level, with same status, helping each other • Intentional: different from being a friend because of intentionality behind it • No expectations: Meet people where they are • Everyone has something to give – create space for that • Amplify strengths • Recognize and encourage people's agency • Credit individuals with capacity to care for themselves • Recognize that we move back and forth on continuum of wellness – if "not in a place to do this today," take it to group and seek support • Base plan on what people need that day – discuss before starting walk • Base support on listening, empathy, being there
Introduce dimensions/types of support in training component walkshops so people can think about what types of support they can offer and receive in walking/rolling groups	Emotional • conversation, connection, forming relationships • encouragement, self-esteem boost • listening, empathy, being there – not fixing, advising • care about story of what happened to peers, not about diagnoses Affirmational • guidance, help • peer support leaders experiencing same things as peer support participants Informational • knowledge of resources Instrumental • people with lived experience creating opportunities for other people with lived experience
WALKING	
Engage people who find physical	• walking is what brings people together – rather than illness, transition,

activity difficult/face barriers to physical activity, and build capacity step by step	challenge • brings together more diverse range of people – positive community building • also empowering – to be "walker," rather than identified and stigmatized by your illness
Ask program participants "How does walking make you feel?" and be prepared to probe or share needs assessment findings.	• reduces social isolation, distress • improves communication skills • builds relationships • entry point into more intense physical activities, in less isolated settings (e.g. gym) • motivated by commitment to other group members • more enjoyable than exercising alone
Integrate planning advice into walkshops	• there are multiple possible strategies to include people walking at different paces • there are multiple formats/agendas possible • 20 mins of exercise with music, before starting walk, then walk for 40mins • breakfast together before walk • coffee together after walk • end walk at park where people can rest • Allow for discovery
GREEN SPACE	
	Integrate green space into walkshops and walking groups, as combined with physical activity it engages participants, reduces their distress and need for support, and builds their self-confidence.

ALEXA YAKUBOVICH: TRAJECTORIES OF EXPOSURE TO NEIGHBOURHOOD DEPRIVATION IN CHILDHOOD AND WOMEN'S EXPERIENCES OF INTIMATE PARTNER VIOLENCE IN ADULTHOOD: A MULTI-METHOD ANALYSIS OF A UK BIRTH COHORT

Supervisor: **Dr. Patricia O'Campo**

Background: Intimate partner violence (IPV) is the most common form of violence perpetrated against women. Although commonly hypothesised, the longitudinal relationship between neighbourhood disadvantage and IPV against women has never been investigated prospectively outside the United States.

Methods: We used data from the Avon Longitudinal Study of Parents and Children in the UK, which followed our target sample, 7,219 women, from birth and their mothers. At age 21, 2,128 participants self-reported their experiences of physical, psychological, or sexual IPV since age 18. Participants' mothers reported on family-level socioeconomic characteristics (e.g., income) at ten time points from baseline (gestation) until children were 18 years old. Participants' exposure to neighbourhood-level deprivation was measured at each time using the Indices of Multiple Deprivation. We estimated the effect of cumulative exposure to greater neighbourhood-level deprivation on the risk of experiencing IPV using marginal structural models with stabilised inverse probability weights, accounting for time- varying confounding by socioeconomic indicators and sample attrition. To investigate whether different trajectories of exposure to neighbourhood deprivation over childhood had greater impacts on IPV in adulthood, we used longitudinal latent class analysis.

Results: A one-unit increase in cumulative exposure to more severe neighbourhood deprivation was associated with a 62% increase in participants' frequency of IPV experiences (IRR=1.62, 95% CI 1.11–2.37) and a 36% increase in their risk of experiencing any IPV (RR=1.36, 95% CI 1.01–1.85). Substantial exposure to greater neighbourhood deprivation at any point over childhood was associated with higher odds of experiencing IPV in adulthood relative to no or minimal exposure (OR=1.41-1.56).

Conclusions: Our findings suggest that cumulative exposure to greater neighbourhood-level deprivation over the first 18 years of life increased women's risk of experiencing IPV in early adulthood. Future studies should test this effect across contexts, including underlying mechanisms, and evaluate preventive strategies that target structural disparities.

JASON LO HOG TIAN: BREAKING THE STIGMA BARRIER: SOCIAL DETERMINANTS OF HEALTH AND THEIR IMPACT ON STIGMA IN PEOPLE LIVING WITH HIV

Supervisor: **Dr. Sean Rourke**

Introduction and Causes for Concern: Experiences of HIV-related stigma and discrimination remain incredibly high in Canada, causing significant stress and negatively affecting the health and well-being of people living with HIV. There is little known about how the social determinants of health are related to experiences of HIV stigma and the ways they can impact health outcomes and access to healthcare services. Understanding these dimensions will allow for more effective treatment as well as inform the creation of initiatives aimed at overcoming HIV stigma.

Scope and Methods: Our study recruited 700 participants in Ontario by ethno-cultural groups, sexual orientation, and socioeconomic status to ensure adequate representation of people with HIV. Trained peer research assistants living with HIV administered the "HIV Stigma Index", designed by people with HIV to measure the nuanced effects that stigma has on their everyday functioning. Validated measures of health risk factors and buffering mechanisms were included in order to accurately assess how they interact with stigma. These constructs were inputted stepwise into a multiple regression model to determine which factors are the main determinants of overall stigma levels.

Results: Multiple regression analysis showed that depression, social support, and resiliency impacted stigma above everything else, with each of the three having about an equal impact on stigma levels. These results suggest that addressing depression is one of the key targets for stigma intervention in addition to ensuring strong social support and increasing resiliency.

Conclusions: This study sheds light on some of the key factors we should focus on and how they interact to alter levels of stigma. Targeting these factors will allow people with HIV to efficiently buffer against the negative effects of stigma and improve interaction with the healthcare system. By decreasing stigma, we will be one step closer to ending the HIV epidemic in Canada.

Poster Presentations

AGNES SEBASTIAN: EFFECT OF GENETICS CLINICAL DECISION SUPPORT TOOLS ON HEALTHCARE PROVIDERS' DECISION MAKING: A SYSTEMATIC REVIEW

Supervisor: **Dr. Yvonne Bombard**

Introduction: While the role of genetics in healthcare is growing, the number of geneticists and genetic counselors remains limited. Thus, non-genetics healthcare providers will increasingly need to manage genetics-related patient care, which can be challenging and unfamiliar. Clinical decision support (CDS) tools are a potential solution because they provide specific risk assessment or management recommendations. This systematic review evaluated whether non-genetics clinicians changed genetics-related patient management when using CDS tools, compared to standard care without CDS.

Methodology: A comprehensive search in Medline, Embase and CINAHL from database inception to March 2019 plus handsearching yielded 2265 articles. Two independent reviewers screened abstracts and full texts for studies comparing management outcomes when non-genetics clinicians used a CDS tool in actual patient care. For the 9 articles included in the final review, effect sizes were calculated and a narrative synthesis was performed. Quality assessment was completed but did not affect study inclusion.

Results: CDS tools focused mainly on pharmacogenetics or cancer, with two tools on thrombophilia and one on cystic fibrosis. CDS tools had a small positive effect on changes in management that was statistically significant compared to pre-implementation but not significant compared to controls (Fig 1). Limitations included low statistical power, sources of bias in the comparative data such as no controls or blinding, and significant heterogeneity of interventions. Certain predictors of CDS tool success, such as an active design and integration into the clinical workflow, were absent in almost all tools.

Conclusions: This systematic review is the first to review quantitative evidence on genetics CDS tools used by non-genetics providers. CDS tools show promise because a small effect on changes in management is observed, but more studies explicitly evaluating tools and bigger sample sizes are needed to establish whether CDS tools will truly support non-genetics clinicians in the genomic age.

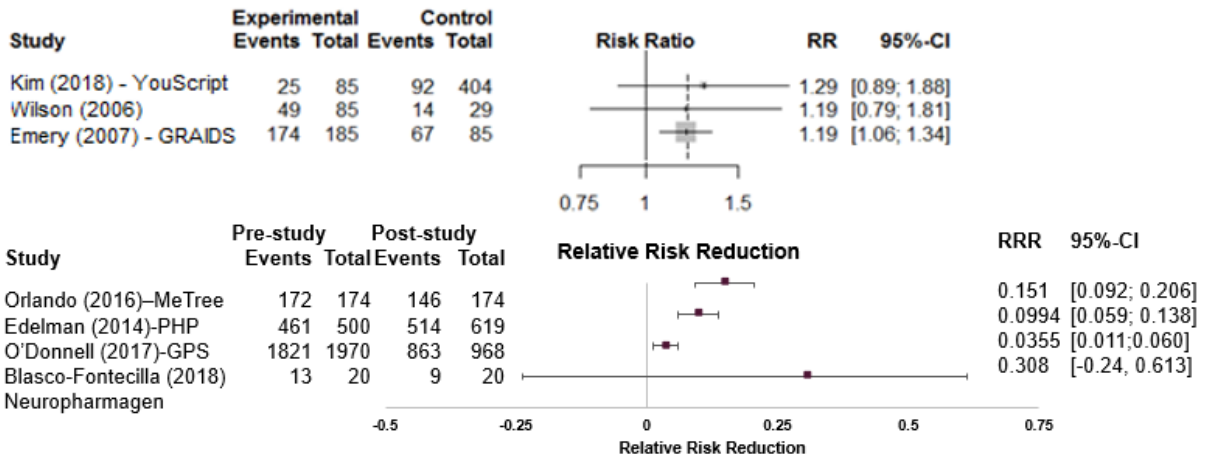


Fig 1: *above* Forest plot of effect size (risk ratios) for the 3 RCTs comparing changes in management between experimental (CDS) and control (no CDS) groups. The effect size across the 3 studies is positive but not consistently significant (the 95% CI crosses 1 for two of the 3 studies).

below Forest plot of effect size (relative risk reduction) for the 4 pre-post studies comparing changes in management pre- and post- implementation of the CDS tool. Overall, CDS tools seem to have a positive effect on changes in management after implementation with three of the tools showing a statistically significant effect (the 95% CI does not cross 0). The Neuropharmagen study does not show a statistically significant effect although this may be due to the small sample size of just 20 patients.

AYLIN VISRAM: ELUCIDATING THE ROLE OF SGLT2 INHIBITOR DAPAGLIFLOZIN IN RIGHT VENTRICLE AND PULMONARY REMODELING

Supervisor: **Dr. Kim Connelly**

Introduction: While 'pure' right heart failure accounts for 2-5% of all heart failure (HF) hospitalisations, many patients with primary right ventricle (RV) failure have secondary left ventricle (LV) failure and present as biventricular disease. Although often considered separately, the structure and function of the LV and RV are inextricably linked. From a structural viewpoint, the two chambers not only share a common septum and pericardial space but as revealed by diffusion tensor MRI also share myocyte bundles that cross between the left and right ventricles. HF might be better considered a biventricular disease whereby LV dysfunction leads to RV dysfunction and vice versa. Dapagliflozin is a sodium glucose linked transporter 2 (SGLT2) inhibitor that has recently been shown to improve heart function in the setting of diabetes. However, the effect of dapagliflozin on HF in a non-diabetic setting is unknown. We hypothesize that by reducing excess fluid volume, the SGLT2 inhibitor dapagliflozin will reduce RV chamber dilatation and wall tension, thereby leading to a reduction in RV hypertrophy and improved function. As such, dapagliflozin will ameliorate RV dysfunction due to both pressure and volume overload.

Methods: Male Fischer rats weighing 200-220 grams will be subjected to either sham or transverse aortic constriction (TAC) surgery, to induce HF. Following surgery, rats will receive either vehicle, dapagliflozin or perindopril, which will serve as a positive control. At end study, rats will undergo measurement for biochemistry, echocardiography and cardiac catheterization.

Expected Results: We expect rats that have underwent TAC to develop pulmonary hypertension, RV hypertrophy, increased right ventricle end diastolic pressure, and lung fibrosis. We expect dapagliflozin, by inducing osmotic diuresis and reducing particular hypertrophy to prevent deleterious ventricular and pulmonary remodeling, thus providing a novel therapy for primary RV failure, as well as RV failure secondary to left sided HF.

CHIRAG VASWANI: THE REGULATION OF OCCLUDIN MRNA BY MIR193B-5P IN INFLUENZA INDUCED ARDS

Supervisor: **Dr. Claudia dos Santos**

Introduction: The leading cause of mortality in critically ill patients from seasonal and pandemic influenza strains is ARDS. Emerging evidence suggests host-derived cellular miRNAs play a critical role in lung injury, alveolar-capillary membrane integrity, and host responses to viral infections. We identified a role for miRNA-193b in the innate immune response to bacteria and the regulation of tight-junction protein (occludin) in experimental models of pneumonia. We advance current knowledge by exploiting the role of this microRNA in barrier function, viral infection and host anti-viral responses in a murine model of H1N1.

Methods: Wild type mice (C57Bl/6J, 10-14 weeks) were infected with H1N1 treated a miR193b-5p inhibitor (INH) versus placebo delivered on day 4 post-infection. Body weights and temperatures were collected. Lungs were harvested on day 8 to assess for: histology, bronchoalveolar lavage fluid cell counts and differential, membrane permeability, and viral load. In vitro, infected Beas2b cells were treated with the miR-193b-5p inhibitor or mimic. Cells were transfected with the siRNA against occludin or scrambled control. Transcripts and miR were evaluated by qRT and digital droplet PCR.

Results: Intranasal infection with H1N1 results in increased pulmonary inflammation, lung edema, increased levels of miR193b-5p (20-fold) and decreased expression of occludin (>50%) that peak at day 5 days post-infection. Reporter construct demonstrates miR-193b binds specifically to the 3'UTR of occludin. Inhibition of miR193b-5p mitigates H1N1-induced lung injury, edema formation, viral load, and anti-viral Interferon β and Interferon Regulated Genes expression. In vitro, silencing of occludin results in increased viral load and dysregulation of the host antiviral response.

Conclusion: MiR-193b-5p plays a critical role in regulation of occludin, tight junction function and virus induced lung injury. Inhibition of miR-193b-5p results in decreased lung injury, inflammation, and viral load.

Supported by the Canadian Institutes of Health Research (Grant # MOP-130331 and MOP-106545 to CCDS).

DANIEL HAN: EXAMINING THE ROLE OF ADIPOCYTE YAP IN REGULATING GLUCOSE TOLERANCE AND OBESITY-ASSOCIATED INSULIN RESISTANCE

Supervisor: **Dr. Cynthia Luk**

Introduction: Obesity and its associated metabolic abnormalities, including type 2 diabetes and its precursor, insulin resistance, are rapidly expanding global epidemics with considerable morbidity and mortality. Studies suggest that adipose tissue hypertrophy leads to fibrosis and metabolically dysfunctional adipose tissue. Furthermore, increased fibrosis formation due to obesity is associated with insulin resistance underlying type 2 diabetes. Yes-associated protein 1 (YAP) is a transcriptional coactivator of the Hippo pathway that have been implicated in the development of lung, liver, and kidney fibrosis. However, the role of YAP in developing adipose tissue fibrosis leading to glucose intolerance and insulin resistance has been understudied.

Methods: Adipocyte-specific YAP knockout and control mice were generated using the adiponectin (adipoq) promoter-driven Cre recombination system. YAP knockout (adipoqCre+YAP-/-) and control littermate (adipoqCre+YAP+/+) mice were placed on standard chow or high-fat diet (HFD) for 12 weeks to mimic basal and metabolic stress conditions. Mice were monitored biweekly for weight, random blood glucose, and 8-hour fasting blood glucose levels. Glucose tolerance and insulin resistance in both groups were assessed by intraperitoneal glucose tolerance test and insulin tolerance test. At the end of 12 weeks, adipose tissues and other glucose-responsive tissues, such as liver and muscle were harvested.

Results: The expression of YAP increased in both subcutaneous and visceral white adipose tissues of mice fed an HFD compared to the control group. Random blood glucose and 8-hour fasting blood glucose levels were lower in the adipoqCre+YAP-/- group compared to the control group in both types of diet. Conversely, insulin sensitivity only improved in the adipoqCre+YAP-/- group on HFD compared to the adipoqCre+YAP+/+ mice.

Conclusion: Our data suggest that knocking out adipocyte-specific YAP in the context of obesity may limit adipocyte hypertrophy and improve glucose tolerance. Further research is needed to determine the role of YAP in developing adipose tissue fibrosis.

DANIELLE LEE: EFFECT OF IMPORTANT FOOD SOURCES OF FRUCTOSE-CONTAINING SUGARS ON NON-ALCOHOLIC FATTY LIVER DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS OF CONTROLLED TRIALS

Supervisor: **Dr. John Sievenpiper**

Objectives: To assess the effects of important food sources of fructose-containing sugars on non- alcoholic fatty liver disease (NAFLD) risk measures at different levels of energy control.

Design: Systematic review and meta-analysis of controlled intervention studies. DATA sources: Medline, Embase, and the Cochrane Library up to 9 January 2019.

Eligibility criteria for selecting studies: Human controlled intervention studies with a ≥7-day follow-up assessing the effect of different food sources of fructose-containing sugars on markers of NAFLD. Four study designs were prespecified on the basis of energy control: substitution studies (sugars in energy-matched comparisons with other macronutrients), addition studies (excess energy from sugars added to diets), subtraction studies (energy from sugars subtracted from diets), and ad libitum studies (sugars freely replaced by other macronutrients without control for energy). Outcomes were intrahepatocellular lipid (IHCL), alanine aminotransferase (ALT), and aspartate aminotransferase (AST).

Data extraction and synthesis: Two independent reviewers extracted relevant data and assessed risk of bias. Data were pooled by the generic inverse variance method using random effects models and overall certainty of the evidence assessed by the grading of recommendations, assessment, development, and evaluation (GRADE) approach.

Results: 18 studies (n=440) were included. In addition trials, there was a significant increase of IHCL (MD=0.44%, 95% confidence interval (CI): 0.26, 0.61) and AST (MD=1.74 U/l, 95% CI: 0.24, 3.24), but not ALT. Fructose showed no effect in substitution or subtraction trials on any of the NAFLD risk markers.

Conclusions: Energy control appears to mediate the effect of fructose-containing sugars. Most studies used SSBs as an intervention, thus limit the ability to assess differences in food sources of fructose-containing sugars. Certainty in these estimates is moderate to very low. Thus, more high quality randomized controlled trials are needed.

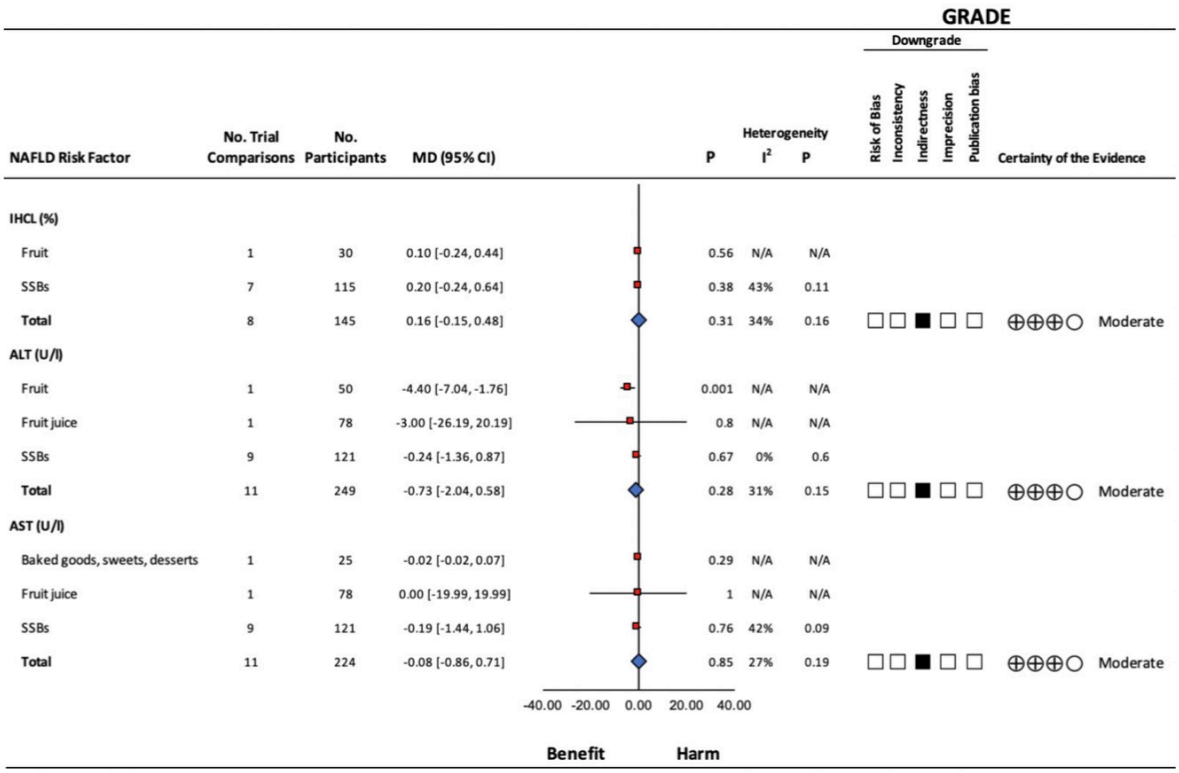


Figure 1. Summary plot of the effect of fructose-containing food sources on IHCL (%), ALT (U/l), and AST (U/l) in substitution trials. Pooled effect estimates for each food source are represented by red squares and pooled effect estimates for the overall effect of fructose-containing sugars are represented by blue diamonds. P-values were determined using random effects modelling in each systematic review and meta-analysis. Between-study heterogeneity was assessed by the Cochran Q statistic, where $P < 0.10$ is considered statistically significant, and quantified by the I^2 statistic, where $I^2 \geq 50\%$ is considered evidence of substantial heterogeneity. The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) of controlled trials are rated as "High" certainty of evidence and can be downgraded by five domains and upgraded by three domains. The filled black squares indicate downgrade for each outcome. ALT=alanine aminotransferase, AST=aspartate aminotransferase, CI=confidence interval; GRADE=Grading of Recommendations, Assessment, Development and Evaluation, IHCL=intrahepatocellular lipid, MD=mean difference.

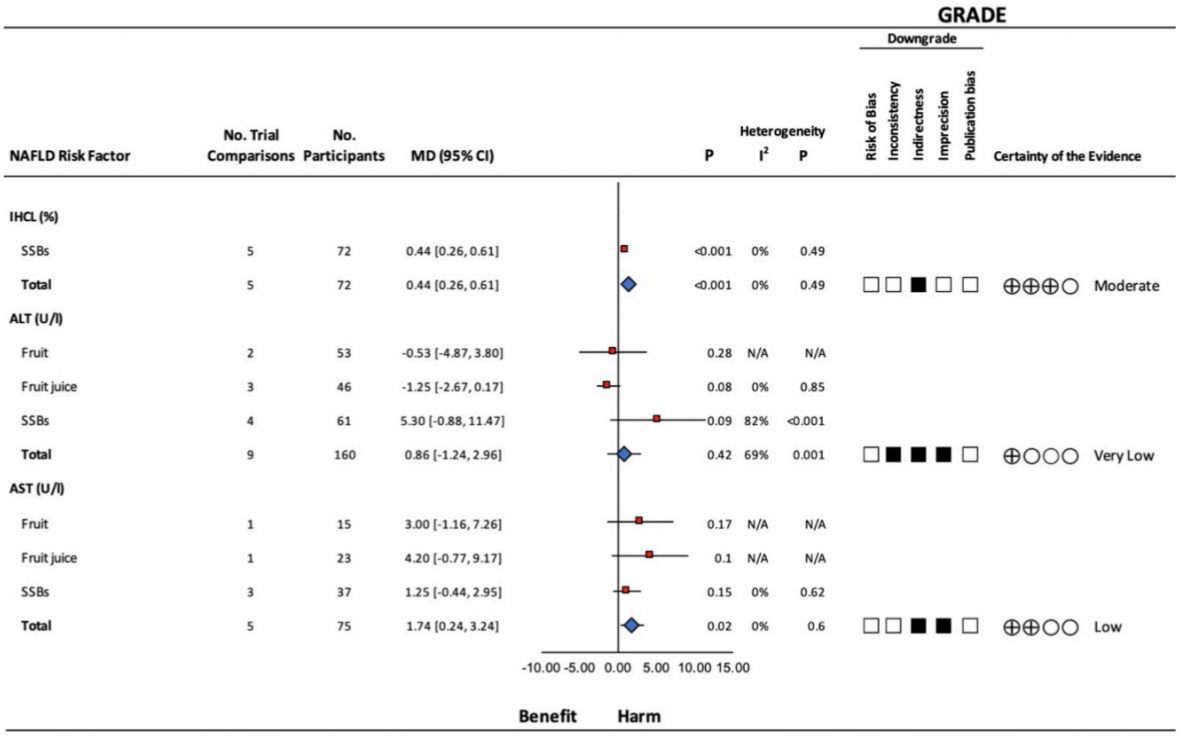


Figure 2. Summary plot of the effect of fructose-containing food sources on IHCL (%), ALT (U/l), and AST (U/l) levels in addition trials. Pooled effect estimates for each food source are represented by red squares and pooled effect estimates for the overall effect of fructose-containing sugars are represented by blue diamonds. P-values were determined using random effects modelling in each systematic review and meta-analysis. Between-study heterogeneity was assessed by the Cochran Q statistic, where $P < 0.10$ is considered statistically significant, and quantified by the I^2 statistic, where $I^2 \geq 50\%$ is considered evidence of substantial heterogeneity. The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) of controlled trials are rated as "High" certainty of evidence and can be downgraded by five domains and upgraded by three domains. The filled black squares indicate downgrade for each outcome. ALT=alanine aminotransferase, AST=aspartate aminotransferase, CI=confidence interval; GRADE=Grading of Recommendations, Assessment, Development and Evaluation, IHCL=intrahepatocellular lipid, MD=mean difference.

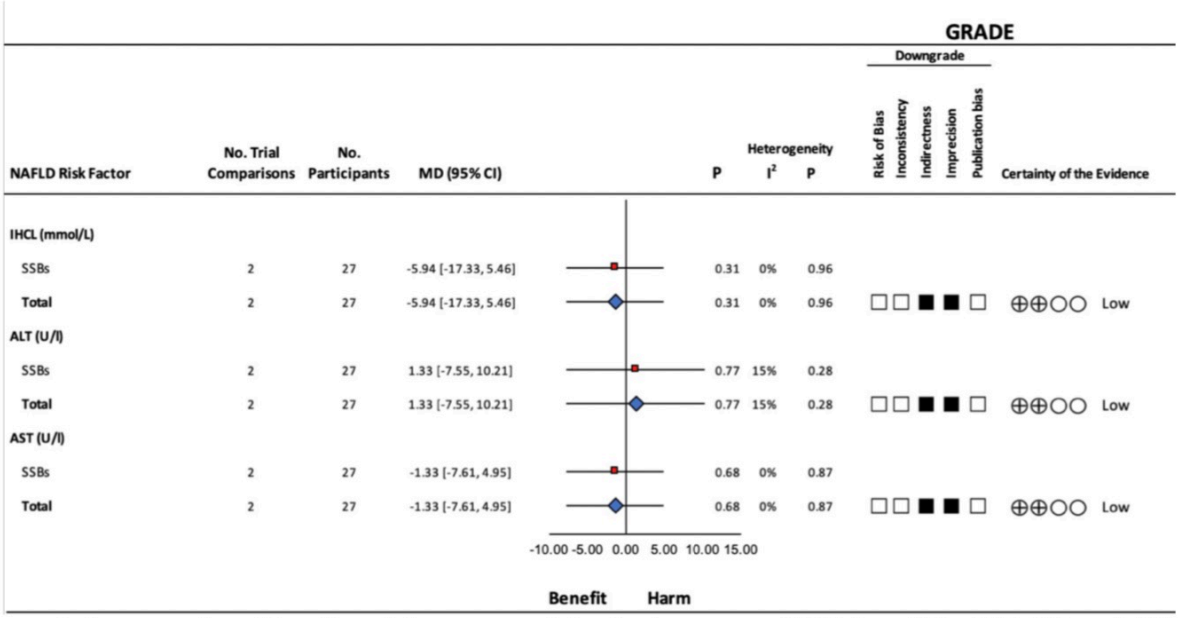


Figure 3. Summary plot of the effect of fructose-containing food sources on IHCL (mmol/L), ALT (U/l), and AST (U/l) levels in subtraction trials. Pooled effect estimates for each food source are represented by red squares and pooled effect estimates for the overall effect of fructose-containing sugars are represented by blue diamonds. P-values were determined using random effects modelling in each systematic review and meta-analysis. Between-study heterogeneity was assessed by the Cochran Q statistic, where $P < 0.10$ is considered statistically significant, and quantified by the I^2 statistic, where $I^2 \geq 50\%$ is considered evidence of substantial heterogeneity. The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) of controlled trials are rated as "High" certainty of evidence and can be downgraded by five domains and upgraded by three domains. The filled black squares indicate downgrade for each outcome. ALT=alanine aminotransferase, AST=aspartate aminotransferase, CI=confidence interval; GRADE=Grading of Recommendations, Assessment, Development and Evaluation, IHCL=intrahepatocellular lipid, MD=mean difference.

FEI RODNEY AU-YEUNG: IMPORTANT FOOD SOURCES OF FRUCTOSE-CONTAINING SUGARS AND FASTING LIPIDS: A SYSTEMATIC REVIEW AND META-ANALYSIS OF CONTROLLED FEEDING TRIAL

Supervisor: **Dr. John Sievenpiper**

Background: Sugar-sweetened beverages are associated with cardiovascular disease. Whether this association is mediated by blood lipids and holds for other important sources of fructose-containing sugars is unclear. To address this question, we conducted a systematic review and meta-analysis of the effect of food sources of fructose-containing sugars on established lipid targets using GRADE.

Methods: MEDLINE, EMBASE, and Cochrane Library were searched through March 9, 2019. We included controlled feeding trials ≥7-days assessing the effect of different food sources of fructose-containing sugars on fasting blood lipids at 4 levels of energy control: substitution (energy-matched comparisons); addition (energy from sugars added to diet); subtraction (energy from sugars subtracted from diet); or ad libitum (energy from sugars freely replaced). Two independent reviewers extracted data and assessed risk of bias. Data were pooled using generic inverse variance and expressed as mean differences (MDs) with 95% confidence intervals (CIs). Overall certainty of the evidence was assessed using GRADE.

Results: 81 substitution (n=2848), 27 addition (n=1453), and 7 subtraction trials (n=1023) met eligibility criteria. No ad libitum trials were identified. In substitution and subtraction trials, there was no effect on LDL (mmol/L), HDL, non-HDL, total cholesterol, or triglycerides on total fructose-containing sugars or individual food sources. In addition trials, fruit juice reduced LDL (-0.19 [-0.34,-0.03]), non-HDL (-0.26 [-0.39,-0.14]), and total cholesterol (-0.19 [-0.28,-0.09]), increased HDL-cholesterol (0.07 [0.02,0.12]), with no effect on triglycerides (-0.03 [-0.10,0.05]). No other effects were observed in addition trials for other food sources. The overall certainty of evidence was “moderate” for fruit juice in addition trials and mixed sources for all other energy controls and “low” for all other comparisons.

Conclusions: Fructose-containing sugars do not have an adverse effect on established lipid targets irrespective of energy control or food source. Further research is needed to improve our estimates.

Protocol registration: ClinicalTrials.gov Identifier, NCT02716870.

Funding: Diabetes Canada, CIHR, PSI Foundation, B&B Diabetes Centre, Toronto3D foundation.

GURLEEN KAUR: CD44 ANTIBODIES – A RECOMBINANT IVIG ALTERNATIVE MEDIATES ITS ANTI-INFLAMMATORY ACTIVITY BY FC GAMMA RECEPTOR INHIBITION

Supervisor: **Dr. Alan Lazarus**

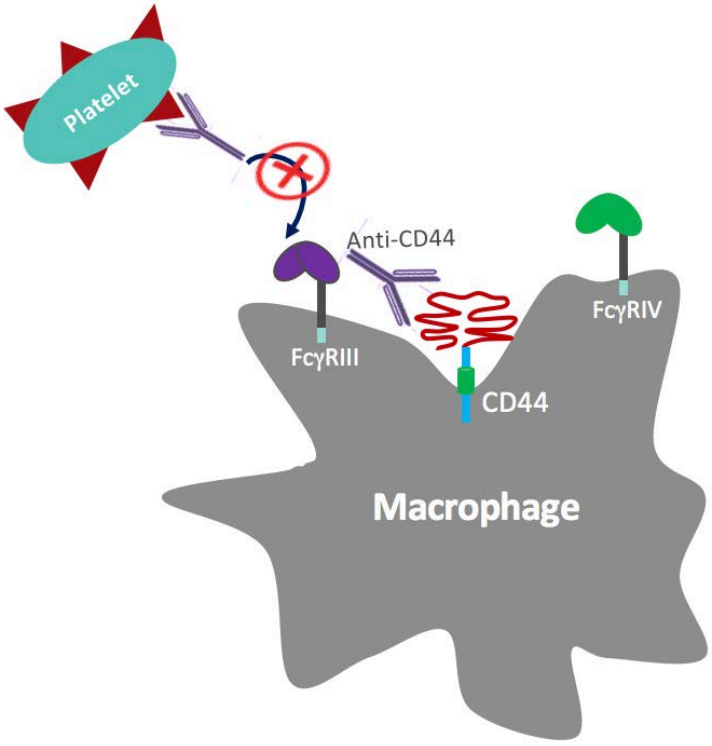
Introduction: Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder where platelet destruction is thought to occur due to autoantibody-sensitized platelets, triggering Fc gamma receptor (FcγR)-mediated phagocytosis. Since Canada is not self-sufficient in manufacturing intravenous immunoglobulin (IVIg), an effective treatment for ITP, a replacement is desired. Using a murine model, we demonstrated that antibodies targeting CD44, can ameliorate ITP at a 3-log fold lower dose than IVIg. This may occur through inhibition of phagocytosis process; however, the exact mechanism remains unknown. We hypothesize that CD44 antibodies may bind and inactivate the FcγR pathway of platelet destruction. Different IgG subtypes bind to specific subclasses of activating FcγRs; if our hypothesis is correct, anti-CD44 should be therapeutic if able to bind the same subclasses of FcγRs as anti-platelet antibody.

Methods: Macrophages were pre-treated with a CD44 antibody, washed, then exposed to opsonized platelets. The activity of intact CD44 antibodies was compared to Fc inactivated versions: deglycosylated and F(ab')2 fragments, to determine the significance of the Fc region in vitro, and in vivo using a murine antibody-mediated ITP model.

Results: Macrophages treated with intact CD44 antibodies resulted in inhibition of platelet phagocytosis. In comparison, CD44 antibodies failed to inhibit phagocytosis when deglycosylated or made into F(ab')2 fragments. Mice treated with anti-CD44 also demonstrated a requirement for a functional Fc region in successful ITP amelioration. Furthermore, anti-CD44 (murine IgG1) could only inhibit in vitro phagocytosis, and ameliorate ITP in vivo when an IgG1 anti-platelet was utilized, as they are both known to bind only FcγR3. Changing the subtype of anti-platelet antibody to an IgG2a (binds FcγR1,3, 4) overcame this restrictive CD44 effect.

Conclusion: These results suggest that CD44 antibodies protect against platelet destruction by binding and inhibiting the activating FcγR pathway (using their Fc region) required for the phagocytosis of opsonized platelets.

Hypothesized mechanism of CD44 antibodies in ITP amelioration.



GURRATTAN CHANDHOKE: SURGEON MANAGEMENT PREFERENCES FOR PATELLA FRACTURES IN ELDERLY, LOW-DEMAND PATIENTS: A CROSS-SECTIONAL SURVEY

Supervisor: **Dr. Aaron Nauth**

Background: There is currently insufficient evidence to support any one treatment for patella fractures in elderly (≥65 years), low-demand patients. Given that older populations often have numerous comorbidities and varying functional needs, treatment algorithms are not always applicable. This study aimed to understand current management practices and surgeon considerations when treating patellar fractures in this population.

Methods: A 37-item electronic survey inquired about surgeon preferences for managing different fracture patterns, operative and non-operative indications, and complication rates. Items were generated from literature and feedback from orthopaedic surgeons. Members of the Orthopaedic Trauma Association and Canadian Orthopaedic Association were recruited to complete the survey. Results were summarized as proportions and stratified for analysis.

Results: Of the 115 surgeons who participated in the survey, 82% practiced in Canada. There was no consensus in the preferred treatment of displaced fractures in elderly, low-demand patients with an intact extensor mechanism: 34% preferred open reduction internal fixation (ORIF) with Kirschner wires and tension band wiring (TBW), 26% preferred screws and TBW, and 15% preferred non-operative management. Practice location and years of experience did not significantly impact treatment approach; however, surgeons ≤40years of age were less likely to treat displaced transverse, comminuted, and superior/inferior pole fractures operatively in comparison to surgeons >40years of age ($p<0.05$). An intact extensor mechanism was the factor most strongly influencing treatment decision, as indicated by 88% of surgeons, followed by compromised soft tissue. Majority of respondents believed that complications occurred in less than 20% of all cases, with symptomatic hardware and knee stiffness as the most common operative and non-operative complication, respectively.

Conclusion: There remains a lack of consensus surrounding the management of displaced fractures in elderly, low-demand populations with an intact extensor mechanism. Further studies are required to establish standards for managing these fractures in an aging population.

HANNA WABNITZ: ANTIGEN LOSS IS IMPORTANT FOR THE ABILITY OF ANTIBODIES TO INDUCE ANTIBODY-MEDIATED IMMUNE SUPPRESSION RELEVANT TO HAEMOLYTIC DISEASE OF THE FOETUS AND NEWBORN

Supervisor: **Dr. Alan Lazarus**

Introduction: Haemolytic disease of foetus and newborn (HDFN) is a severe, potentially fatal, neonatal disorder caused by the transfer of maternal alloantibodies into the foetus' circulation where they destroy foetal red blood cells (RBCs). Since the 1970s, HDFN can be effectively prevented though the administration of pooled, plasma-derived anti-D by a mechanism known as antibody-mediated immune suppression (AMIS). The AMIS mechanism is poorly understood but it has been proposed that erythrocyte clearance plays a major role. However, accumulating evidence has indicated that RBC clearance is not required for AMIS and has suggested antigen loss as a potential mechanism of action instead. The aim of this work was to determine whether the induction of AMIS in a mouse model of HDFN was more closely related to the loss of the antigen or the clearance of RBCs.

Methodology: Transgenic HOD mice expressing the HOD antigen composed of hen egg lysozyme (HEL), ovalbumin (OVA) and the human Duffy transmembrane protein were bled as a source of foreign RBCs. The RBCs were subsequently labelled with the fluorescent dye PKH26 and transfused into wild-type C57BL/6. 24 hours later 3 antibodies directed against the HOD antigen were injected and blood was collected at 0, 2, 24 and 48 hours to track the presence of the HOD cells. Antigen loss was detected via Flow cytometry and IgM and IgG responses were measured by ELISA as an indication of AMIS.

Results: Antigen loss was observed in 3/3 cases of AMIS induction by the different antibodies, while only one out of the 3 antibodies which caused AMIS also induced the clearance of the labelled HOD-RBCs.

Conclusions: This work shows that RBC clearance is not essential for AMIS induction. The antibodies' ability to induce antigen loss rather than RBC clearance was more closely related to its ability to induce AMIS.

IZABELA SOCZYNSKA: PLANT-BASED MILK CONSUMPTION IN YOUNG CHILDREN

Supervisor: **Dr. Jonathon Maguire**

Background: Parents are increasingly replacing cow milk with plant-based milks (e.g. soy, almond, rice, coconut) in children's diets, with 13% of Canadian children now consuming these beverages on a daily basis. Little is known about the reasons for the increase in plant-based milk consumption among young children and perceptions towards plant-based milks, particularly regarding nutritional content, among parents and physicians.

Objectives: The objectives of the study are to: (1) to explore parents' and physicians' knowledge and perceptions toward plant-based milks in children's diets; (2) to determine the *type* of plant-based milks parents provide to their children and what milk recommendation primary care physicians provide to families of children who cannot consume cow milk; (3) to understand the *reasons* for the rise in childhood plant-based milk consumption (e.g. lactose intolerance, vegetarian lifestyle) and; (4) to assess parent and physician willingness to provide fortified soy milk as a replacement for cow to children to determine the feasibility of conducting a clinical trial.

Methods: A mixed methods study will be conducted. Online questionnaires will first be used to understand current practices, knowledge and perceptions about plant-based milks for children among parents and physicians followed by semi-structured interviews to gain a better understanding of the rationale behind the questionnaire responses.

Results: The results of this study will provide a better understanding on the factors that influence parents' and physicians' decisions about which milk to provide to young children, and the driving factors that have contributed to the rise in childhood plant-based milk consumption.

Conclusion: This study will fill a gap in the current literature about the reasons behind the increase in plant-based milk consumption and shed light on the profiles of children who consume these beverages, which will help to inform future research, practice guidelines and public nutrition policy.

JENNIFER LEE: RELATION OF CHANGE OR SUBSTITUTION OF ARTIFICIALLY SWEETENED BEVERAGES ON CARDIOMETABOLIC OUTCOMES: A SYSTEMATIC REVIEW AND META-ANALYSIS OF PROSPECTIVE COHORT STUDIES USING GRADE

Supervisor: **Dr. John Sievenpiper**

Background: Artificially sweetened beverages (ASBs) are associated with risk of cardiometabolic outcomes. This association is likely due to reverse causality. We conducted a systematic review and meta-analysis on ASBs and cardiometabolic health using change and substitution results that can overcome this limitation.

Methods: MEDLINE, EMBASE, and the Cochrane Library were searched for prospective cohort studies (≥1-year follow-up) that assessed change in ASB intake or substitution of ASB for another sugary-beverage on cardiometabolic outcomes in adults. Independent reviewers extracted data and assessed study quality (Newcastle-Ottawa Scale). Data were pooled using the random-effects model and expressed as mean differences (MDs) or risk ratios (RRs) with 95% CIs. Heterogeneity was assessed (Cochran Q statistic) and quantified (I2 statistic). Certainty of evidence was assessed using GRADE.

Results: Nine studies (395,731 individuals) were included. Increasing 1-serving of ASBs was associated with reduced body weight (MD: -0.07 kg [-0.10 to -0.04]) and waist circumference (MD: -1.35 cm [-2.60 to -0.05]). Substitution of ASBs for SSBs was associated with reduced body weight (MD: -0.47 kg [-0.55 to - 0.39]), risk of diabetes (RR: 0.94 [0.90 to 0.98]), cardiovascular mortality (RR: 0.95 [0.90 to 0.99]) and total mortality (RR: 0.96 [0.94 to 0.98]). Substitution of ASBs for 100% fruit juice was associated with reduced body weight (MD: -0.47 kg [-0.53 to -0.41]) and risk of type 2 diabetes (RR: 0.91 [0.83 to 0.99]). However, substitution of ASBs for water was not associated with body weight or incidence of obesity. The overall certainty of the evidence by the GRADE System was "very low" for all outcomes due to downgrades for indirectness and/or imprecision.

Conclusions: The use of ASBs to substitute excess calories from free sugars may offer relative benefits for cardiometabolic health; however, more studies with robust statistical methods are warranted for evidence-informed public health recommendations.

KAREN FUNG: INHIBITION OF LOW DENSITY LIPOPROTEIN INTERNALIZATION AND TRANSCYTOSIS BY HDL; AN ALTERNATIVE ROLE FOR "GOOD" CHOLESTEROL

Supervisor: **Dr. Greg Fairn & Dr. Warren Lee**

Atherosclerosis results from the build-up of low-density lipoprotein (LDL) cholesterol and immune cells in the arteries leading to their occlusion. High-density lipoprotein (HDL) is believed to reverse this process by removing the arterial cholesterol from the body. Both LDL and HDL reach beneath the artery by crossing the endothelium through Scavenger receptor B1 (SR-B1) mediated transcytosis. ApoAI, the protein exclusively found on HDL, mediates the interaction between HDL and SR-B1. One natural ApoAI variant called Milano (ApoAI-Mil) is of interest because injections of lipidated ApoAI-Mil led to enhanced plaque regression. We hypothesize that the natural ability of ApoAI-Mil to dimerize leads to better interaction with SR-B1. This could result in better inhibition of LDL transcytosis since both HDL and LDL bind to SR-B1 for transcytosis and this could also improve HDL transcytosis so that more HDL will reach the target tissue to extract cholesterol. We took an in vitro microscopy approach to quantify internalization of fluorescently labeled LDL in coronary endothelial cells. The ApoAI variants were also lipidated to form fluorescent HDL-like particles (DiI-DMPC-WT or DiI-DMPC-Mil) to measure its association with SR-B1 overexpressing cells. We observed that recombinant ApoAI-WT can inhibit LDL internalization in coronary endothelial cells. There was also about an 8x-fold increase of DiI-DMPC-WT fluorescence signal in HeLas overexpressing SR-B1 compared to GFP alone. Furthermore, there was an even higher fold increase (~11x) of DiI-DMPC-Mil fluorescence signal compared to GFP alone. Similarly, excess recombinant ApoAI-Mil led to greater inhibition of LDL internalization compared to ApoAI-WT. There was no difference in the amount of DiI-DMPC-WT or -Mil associated with HeLa cells overexpressing Alk1, a LDL-specific transcytosis receptor. Together this suggests that compared to ApoAI-WT, ApoAI-Mil more readily associates with only SR-B1 which may lead to better inhibition of LDL internalization and to more HDL available to remove arterial cholesterol.

KATE RZADKI: IDENTIFYING BARRIERS TO COMPLETION OF ADJUVANT THERAPY IN PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME

Supervisor: **Dr. Sunit Das**

Introduction: This study aims to identify the key factors that are influencing one third of medically eligible newly diagnosed glioblastoma (GBM) patients diagnosed at St. Michael's Hospital to choose to decline or withdraw from the recommended regimen of chemoradiation, the Stupp protocol. We hypothesize that there are underlying factors that influence a GBM patient's decision to decline or withdraw from the Stupp protocol, other than those related to the disease itself, and that these factors fall within Kim et al.'s Conceptual Framework for Individual and Family End-of-Life Decision Making[1].

Methodology: In this mixed methods study, data will be collected from two sources: medical chart review and semi-structured interviews. During the chart review of newly diagnosed GBM patients (n=150), factors common amongst patients who have not completed treatment will be analyzed in order to identify profiles of those at risk of declining or withdrawing from care. Next, semi-structured interviews will be conducted with three groups: newly diagnosed GBM patients who have declined or withdrawn from chemoradiation (n=20), caregivers (n=20), and healthcare providers (n=10). Data collected from the interviews will be analyzed to identify common themes or factors related to the decision to decline or discontinue treatment and the decision-making process.

Results: Chart review and interviews are currently underway and it is anticipated that quantitative analysis will be completed by November.

Conclusions: The results of this study may be used to inform practitioners by identifying barriers to completion of chemoradiation which in turn, may lead to the identification of patient profiles at risk of withdrawing from care. Identifying these risk factors may help in the development of tailored resources that can be used to better support GBM patients in the treatment and decision-making process. The findings from the analysis will also offer supporting or non-supporting evidence to Kim et al.'s framework.

[1] Kim, K., Heinze, K., Xu, J., Kurtz, M., Park, H., Foradori, M., & Nolan, M. T. (2018). Theories of Health Care Decision Making at the End of Life: A Meta-Ethnography. Western Journal of Nursing Research, 40(12), 1861–1884. https://doi.org/10.1177/0193945917723010

LAURA ELLIOTT: ASSOCIATION BETWEEN VEGETARIAN DIET, GROWTH AND MICRONUTRIENT STORES IN EARLY CHILDHOOD

Supervisor: **Dr. Jonathon Maguire**

Introduction: The new Canada food guide has recommended that Canadians increase consumption of fruits, vegetables, and plant-based proteins. Vegetarian diets are becoming increasingly popular among Canadian's, yet few studies have evaluated the relationship between vegetarian diet and childhood growth and nutritional status. Since vegetarian diets can be less energy dense and may have lower micronutrient content, we hypothesized that vegetarian diet may affect childhood growth including lower adiposity and height, and lower micronutrient stores.

Methods: This was a prospective study of healthy children age 6 months to 10 years participating in the TARGet Kids! cohort study. The primary exposure was vegetarian diet, measured by parent report. The primary outcome was BMI z-score. Secondary outcomes were height-for-age z-score, serum ferritin, and serum 25-hydroxyvitamin D. Anthropometric measures and venous blood samples were collected at health supervision visits by trained research assistants. Linear mixed effect modelling was used to determine the association between vegetarian diet, growth, and micronutrient stores.

Results: A total of 8532 children (n = 223 vegetarian) participated. In the adjusted models there was no evidence of an association between vegetarian diet and BMI z-score (p = 0.40, 95% CI: -0.05, 0.12), serum ferritin (p = 0.93, 95% CI: -3.31, 3.60), or 25-hydroxyvitamin D (p = 0.62, 95% CI: -5.77, 3.46). In the multivariate analysis, vegetarian diet was associated with an average 0.10 lower height-for-age z-score (p = 0.02, 95% CI: -0.18, -0.01), which is equivalent to 0.4 cm for a 3-year-old child.

Conclusion: In this prospective cohort study of children age 6 months to 10 years, evidence of an association between vegetarian diet and lower adiposity or lower micronutrient stores was not found. However, vegetarian diet was associated with slightly shorter stature. Future research is needed to evaluate the potential mechanisms influencing the association between vegetarian diet and childhood height.

LUCAS (JARYD) TE: ENABLING SKELETAL MUSCLE REPAIR AND FUNCTIONAL RECOVERY FOLLOWING DENERVATION-INDUCED INJURY USING ULTRASOUND MEDIATED GENE DELIVERY (UMGD)

Supervisor: **Dr. Jane Batt**

Jaryd Te[1], Michael Kuliszewski[1], Judy Correa[1], Howard Leong-Poi1,[2], Jane Batt[1,3]
[1]Keenan Research Center for Biomedical Science, St Michaels Hospital,[2]Division of Cardiology, St Michaels Hospital,
[3]Interdepartmental Division of Critical Care Medicine, University of Toronto.

Introduction: Skeletal muscle is essential for mobility and its health relies upon innervation. Accidental limb trauma with peripheral nerve injury results in immediate loss of muscle function, muscle atrophy, and development of fibrosis, which can be irreversible if re-innervation is delayed. Over time, permanent functional disability results. Our goal is to use the novel approach of ultrasound-mediated gene delivery (UMGD) to promote the repair and regeneration of denervated muscle and sustain its receptivity to reinnervation.

Methods: We denervate the gastrocnemius and soleus muscles by transecting the tibial nerve in one hindlimb, while the contralateral leg serves as an unoperated control. DNA-containing minicircle plasmids are coupled to cationic microbubbles and are administered intravenously following nerve transection. The ultrasound transducer is swept over the denervated muscles, which cavitates microbubbles and allows minicircle uptake and expression in target cells. Muscle is harvested from 2 weeks to 3 months following nerve transection. Atrophy (muscle weight, myofiber type-specific cross sectional area), vascularity, satellite cell dynamics, Fibro-adipogenic progenitor (FAP) content, and fibrosis progression are determined with morphometry, histology, immunohistochemistry, flow cytometry and Western blotting.

Results: We have established the baseline time course of key denervation-mediated events to determine the optimum timeline for UMGD (Fig. 1). We have developed and optimized a method for identifying Fibro-adipogenic progenitors (FAPs), a population of resident interstitial cells that are presumed to play a role in denervation injury. We have shown that FAPs increase their numbers throughout the first 5 weeks following denervation. We have also conducted a pilot UMGD trial, in which we demonstrate successful minicircle expression for at least 4-days post-delivery.

Conclusions: We have characterized the long-term post-denervation changes in rat gastrocnemius muscle, and demonstrated the influx of FAPs in the context of other post-denervation pathologic features. We also demonstrate the efficacy of UMGD via successful minicircle delivery.

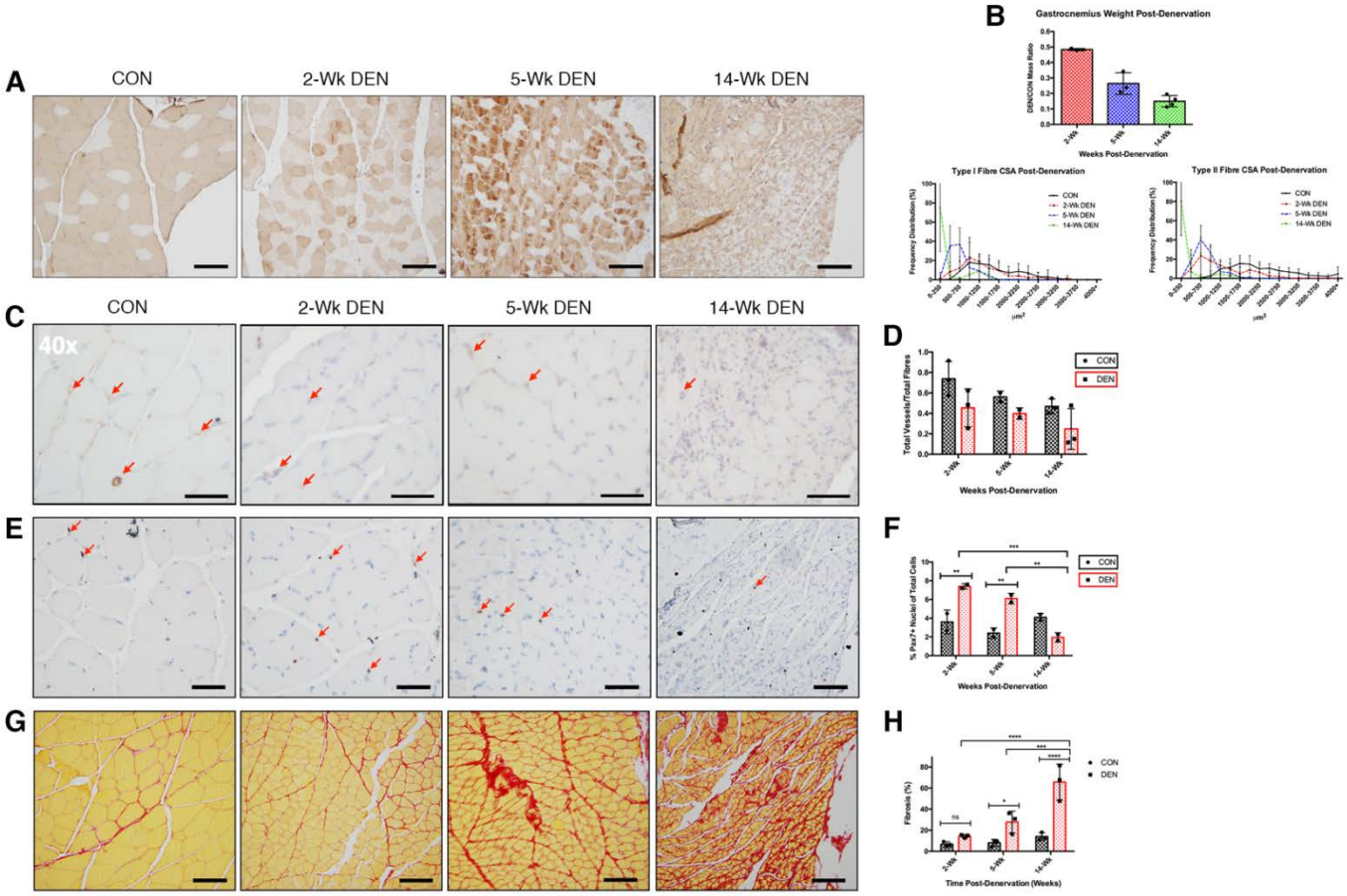


Figure 1. Characterization of long-term denervation injury in the rat.

A) My32 immunostaining of healthy (CON) and denervated (DEN) gastrocnemius sections analyzed at the indicated time points. Type I fibres are unstained (white), while Type II fibres stain brown. Scale bar 100um. **B)** Ratio of denervated to healthy gastrocnemius weights (top) and frequency distribution plots of type I (bottom left) and type II fibre (bottom right) cross-sectional area. **C)** Muscle sections stained for CD31 at indicated time points. Red arrows denote CD31-positive vessels. Scale bar 50um. **D)** Quantification of total vessels divided by total muscle fibres observed in C). **E)** Immunostained sections for Pax7, a satellite cell-specific marker. Red arrows denote Pax7-positive nuclei. Scale bar 50um. **F)** Quantification of percentage Pax7+ nuclei of total nuclei. ** = $p < 0.01$; *** = $p < 0.001$. **G)** Muscle sections stained with picrosirius red (PSR); red denotes areas of collagen. Scale bar 100um. **H)** Quantification of G) showing percentage of fibrotic tissue (n=3). * = $p < 0.05$; **** = $p < 0.0001$. For all experiments, N=4 for 2-week and 14-week timepoints, and N=3 for the 5-week timepoint; values represent mean ± s.d.

Michelle Dubinsky: Role of Hemodynamic-Mediated Endothelial Cell Cis-Acting DNA Element in Regulating Gene Expression Changes

Supervisor: **Dr. Philip Marsden**

Endothelial nitric oxide synthase (eNOS) is a crucial combatant of vascular disease, as it produces the vasodilator nitric oxide. Reduced expression and transcriptional activity of eNOS is a hallmark of endothelial dysfunction, observed in the endothelium overlaying human atherosclerotic lesions. Arterial bifurcations and curvatures are susceptible to atherosclerosis and the endothelial cells (ECs) in these regions experience complex and disturbed (diseased) blood flow patterns, with low shear stress. We have shown that these regions of the mouse aorta display reduced eNOS expression and transcriptional activity. Conversely, when laminar (healthy) flow with high shear stress is present, as in straight segments of the arterial network, eNOS is increased. Investigating the gene regulatory mechanisms underlying vascular EC function in response to hemodynamic forces is crucial in understanding cardiovascular pathologies. EC gene expression patterns are regulated by blood flow through both cis-trans pathways and epigenetic mechanisms. Our objective is to elucidate the role of a major cis-acting DNA element, the shear stress responsive element (SSRE). We hypothesize the SSRE cis-element and its corresponding transcription factors act as regulators of gene expression in response to differential flow conditions. Our lab has mutated the SSRE in vivo and shown that eNOS gene expression is attenuated. This mutation has a differential effect in episomal versus chromatin-incorporated reporters. Through sequence conservation analysis, we have noted the reported SSRE binding motif is only a portion of the conserved nucleotides likely to play a role in shear responsiveness. Mass spectrometry analysis revealed ~200 proteins that bound to an SSRE probe under flow. In silico prediction tools and TF binding from accessible ChIP-seq data were explored for overlap with bound proteins: candidates include Helicase-like Transcription Factor (HLTF), PPAR and ZC3H18. In conclusion, we predict proteins that bind to the SSRE contribute to the atheroprotective phenotype of ECs. This work will provide insights into transcriptional complexes involved in the etiology of atherosclerosis.

MUSKAN GUPTA: IDENTIFICATION OF NOVEL MICRORNAS REGULATING SKELETAL MUSCLE REGENERATION IN SUSTAINED ICUAW

Supervisor: **Dr. Jane Batt**

Intensive care unit-acquired weakness (ICUAW) is a common complication of critical illness characterized by decreased skeletal muscle mass and impaired contractile function that may persist for years after ICU discharge. This can ultimately lead to weakness and permanent physical disability. Our understanding of the mechanisms underlying sustained ICUAW is limited. Our lab has previously shown that survivors of critical illness with irreversible sustained atrophy exhibit reduced muscle satellite cell content, suggesting that their muscles have impaired regeneration capacity, which contributes to sustained ICU acquired weakness.

MicroRNAs (miRs) regulate gene expression in muscle satellite cells and myoblast proliferation and differentiation, thereby influencing the maintenance and self-renewal of skeletal muscle. Therefore, we conducted an integrated miR-mRNA analysis (of quadriceps biopsies from patients with early and sustained ICUAW) to identify dysregulated miR/gene pairs that influenced impaired muscle regeneration in ICUAW, and evaluated their impact on myoblast proliferation and differentiation in vitro. At 6 months post-ICU, distinct miR expression signatures were found to separate patients with significant improvement in muscle mass from those with sustained ICUAW and persistent muscle atrophy. Eight miRs were found to regulate these differentially expressed gene signatures, including miR-490-3p and 744-5p, which we have identified as novel regulators of myogenesis. miR-490-3p overexpression significantly reduced C2C12 myoblast proliferation and induced contact independent myoblast differentiation. miR-744-5p overexpression did not affect myoblast proliferation, but instead attenuated myoblast differentiation. Therefore, miR-490-3p and miR-744-5p are regulators of myogenesis that impact recovery from ICUAW.

NEGAR KHOSRAVIANI: EXPRESSION AND FUNCTION OF DARC IN ENDOTHELIAL CELLS

Supervisor: **Dr. Warren Lee**

Inflammation is the body's defense mechanism against tissue damage and infection. Chemokine receptors expressed on endothelial cells (ECs) of the post-capillary venules present chemokines to leukocytes and support their transmigration across the endothelium. Recently, a new chemokine receptor known as the Duffy Antigen Receptor for Chemokines (DARC) has been identified to be expressed in microvascular EC and it has been shown to play a role in leukocyte diapedesis. However, little is known about the function and mechanism of endothelial DARC due to the loss of expression in ECs, which poses a limitation to studying its function in vitro. Our lab recently discovered that incubation with whole blood for 24 hours was sufficient to induce DARC expression in primary human pulmonary microvascular endothelial cells (HPMEC). Therefore, this leads us to hypothesize that the cellular component of blood and/or its hemodynamics induce DARC expression in ECs of the postcapillary venules. HPMECs incubated with whole blood for 24 hours, increased DARC mRNA and protein levels compared to cells incubated with media, or with plasma isolated from the same blood. To confirm the expression is endothelial specific and not a contribution from erythrocyte DARC, chromatin immunoprecipitation (ChIP) was performed in order to show enrichment of RNA polymerase II (RNA Pol II) at the DARC gene. Significantly higher levels of RNA Pol II were detected in HPMECs incubated with whole blood. Furthermore, we observed loss of DARC mRNA and protein levels as early as 6 hours after washing the blood off HPMECs. This suggests instability of DARC mRNA and protein, and loss of DARC transcription regulatory factors. Therefore, elucidating the mechanism by which whole blood induces DARC expression in ECs is necessary in order to uncover the role of DARC in leukocyte emigration.

NÉMA MCGLYNN: SYSTEMATIC REVIEW AND NETWORK META-ANALYSIS OF NON-CALORIC SWEETENED BEVERAGES VERSUS WATER AND CARDIOMETABOLIC RISK

Supervisor: **Dr. John Sievenpiper**

Background: Health authorities recommend a reduction in sugar sweetened beverages (SSBs). Water is the preferred replacement strategy as there is concern that non-caloric sweetened beverages (NSBs) contributes to increased diabetes risk. To address this concern, we conducted a systematic review and network meta-analysis (SRMA) of randomized controlled trials (RCTs) assessing the effect of NSBs versus water on glycemic control using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach.

Methods: Medline, EMBASE, Cochrane Library was search through March 6th, 2018. We included RCTs ≥ 7 days duration that compared the effect of any two of the three comparators (NSBs, water, SSBs) on HbA1c, fasting plasma glucose (FPG), fasting plasma insulin (FPI) and HOMA-IR. Two reviewers independently extracted relevant data and assessed risk of bias (Cochrane Risk of Bias tool). Data were pooled using random effects network meta-analysis in which mean differences (MD) with 95% confidence intervals (CIs) were synthesized for direct comparisons (NSB vs. Water) with contribution from indirect comparisons (NSB vs. SSB and SSB vs. Water). Heterogeneity was assessed (Cochran Q) and quantified (I2 statistic). The overall certainty of the evidence was assessed using GRADE.

Results: Eligibility criteria were met by 8 RCTs in 796 predominantly overweight/obese participants. Compared with water, NSBs did not show an effect on HbA1c (0.32 [-0.11, 0.74]), FPG (MD, 0.024 [-0.68, 0.114]), FPI (9.18 [-1.94, 20.31]), or HOMA-IR 0.19 [-0.18, 0.56]), and HbA1c (0.32 [-0.11, 0.74]) with evidence of substantial heterogeneity across all outcomes (all I2>50%, p<0.001 from pairwise-analysis). The certainty of the evidence was graded as “high” from FPG, “moderate” for FPI and HOMA-IR with downgrades for serious imprecision and “very low” for HbA1c with additional downgrades for serious indirectness and inconsistency.

RAJIV SANWAL: THE ENDOTHELIAL BARRIER IS NOT RATE-LIMITING TO INSULIN ACTION IN THE MYOCARDIUM

Supervisor: **Dr. Warren Lee**

Diabetes is one of the most prevalent diseases in the world, with 11 million Canadians currently living with diabetes or prediabetes. Its most common form is characterized by the impaired function of the pancreatic hormone insulin. Being an endocrine hormone, insulin must use the vasculature to travel to its target tissues. The exit of insulin from the capillaries requires its passage across a confluent monolayer of endothelial cells, which serves as a barrier between blood and tissue. Whether the endothelium acts as a rate-limiting barrier to insulin transport is controversial, primarily due to the difficulties posed with answering the question using current in vitro and in vivo models.

We hypothesized that if the endothelial barrier is rate-limiting to insulin transport, then increasing endothelial permeability will accelerate insulin action on downstream tissues. To address the question, we developed a murine ex vivo perfusion model which delivers insulin to the heart. Using a syringe pump, we perfused the coronary arteries at a constant rate through retrograde aortic perfusion.

Our model successfully induced a myocardial response to insulin. We demonstrated that both platelet activating factor (PAF) and vascular endothelial growth factor (VEGF) significantly increase cardiac vascular permeability. Despite this increased permeability, insulin action was not accelerated at both 2 and 5 minutes. We then inhibited fluid-phase transport and found no decrease in the rate of insulin action. Finally, we perfused hearts with a tenfold lower dose of insulin and found that PAF does not accelerate insulin action at this dose.

Together, these data indicate that the endothelial barrier is not rate-limiting to insulin action in the myocardium. Efforts to decrease insulin resistance should focus on downstream tissues or regulation of blood flow rather than the transendothelial transport of insulin.

RAZAN TURKI: STRETCH-INDUCED ACTIVATION OF HIPPO SIGNALING IN LUNG MICROVASCULAR ENDOTHELIAL CELLS – A NOVEL MECHANISM OF OVERVENTILATION-INDUCED PULMONARY FIBROSIS

Supervisor: **Dr. Wolfgang Kuebler**

Mechanical ventilation is a mainstay of current therapy for patients with acute respiratory distress syndrome (ARDS). Yet despite its benefits, mechanical ventilation can also cause ventilator induced lung injury (VILI) that may ultimately progress to pulmonary fibrosis. In this study, we aimed to elucidate cellular mechanisms by which mechanical ventilation triggers pulmonary fibrosis. Specifically, we focused on the mechanosensitive transcriptional co-factor TAZ, a key player in the HIPPO-signaling pathway involved in mechanosensation. In the HIPPO pathway, phosphorylation of Large Tumor Suppressor Kinases (LATS) regulates the phosphorylation state of TAZ and thus, its translocation to the nucleus. Despite its emerging relevance in tissue fibrosis, TAZ activation, its underlying mechanisms and downstream profibrotic effects upon mechanical ventilation or its in vitro correlate cyclic stretch have not been addressed. Objectives: To examine the effect of mechanical stretch on the nuclear translocation of TAZ in lung microvascular endothelial cells, and to identify underlying regulatory mechanisms and downstream effects. Methods: To mimic the effects of mechanical overventilation, human pulmonary microvascular endothelial cells (HPMECs) were subjected to stretch regimens at either 5%, mimicking low tidal volume ventilation, or 18% mimicking high tidal volume ventilation at 0.25 Hz for different time intervals. Results: Mechanical stretch induced the release of the profibrotic cytokine TGF-β1 from HPMECs and concomitantly caused translocation of TAZ from the cytosol to the nucleus in a dose- and time dependent manner. Specifically, the nuclear to cytoplasmic ratio (N/C) of TAZ increased after 3h of stretch at 18% compared to the static control whereas stretching these cells at 5% increased TAZ nuclear localization after 6h. Stretch-induced TAZ translocation was not prevented by pharmacological inhibition of JNK or Src kinases, known regulators of the HIPPO kinase LATS2; yet TAZ translocation was blocked by inhibition of Aurora kinases (SNS 314 mesylate; 10 µMol/L). Consistently, inhibition of Aurora kinases decreased LATS2 phosphorylation at Ser83. Conclusion: These data suggest that mechanical stretch activates TAZ and triggers the release of pro-fibrotic cytokines, at least in part, via an Aurora kinase and LATS2-dependent mechanism.

ROBINSON TRUONG: A SYSTEMATIC REVIEW OF THE IMPACT OF CHRONIC TETRACYCLINE CLASS ANTIBIOTICS ON ANTIMICROBIAL RESISTANCE IN HOST NORMAL FLORA

Supervisor: **Dr. Darrell Tan**

Background: There is an emerging interest in daily oral doxycycline for use as a long-term STI pre-exposure prophylaxis (PrEP) in an era of heightened risk of antimicrobial resistance (AMR). To understand how much the use of these antibiotics as PrEP may add to the existing threat of AMR, we are conducting a systematic review of the impacts of chronic oral tetracycline class antibiotics on the development of AMR in normal flora.

Method: We searched through MEDLINE, EMBASE, Cochrane Library electronic databases (1940-2019) and conference proceedings (2014-2019), and included randomized controlled trials that compared adults who took daily oral tetracycline class antibiotics to those who took a placebo, alternative antibiotics or no antibiotics at all. We primarily included articles with various AMR outcomes, such as fold change in tetracycline MIC in flora bacteria. We also included articles that demonstrated tetracycline's impact on cross-resistance and sexually transmitted infection incidences. After all articles were screened by two independent reviewers (RT, VT) based on the inclusion criteria, the included articles were extracted for data analysis and assessed for risk of bias.

Results: Our search strategy yield 5588 potential articles of which 24 articles fulfilled the inclusion criteria. The sample characteristics of these studies include a total n= 2556, age range=11-75yrs, and a mean of 58.6% male participants. Within the 24 articles, 19 interventions were doxycycline, 3 were minocycline and 2 were tetracycline. Interestingly, the majority of studies were on subgingival (13) and gastrointestinal (4) flora. Currently, we are in the process of studying outcomes such as microbiological burden as changes in %resistant isolates during the intervention, and post-intervention AMR burden.

Conclusion: We anticipate that this review will complement current clinical trials of doxycycline PrEP, but will also inform us on AMR trends with tetracycline antibiotics, cross-resistance and STI incidences.

Selena Osman: First Steps to Nanomedicine: Assessing Interactions between Polystyrene Nanoparticles and Albumin Proteins

Supervisor: **Dr. David Cramb**

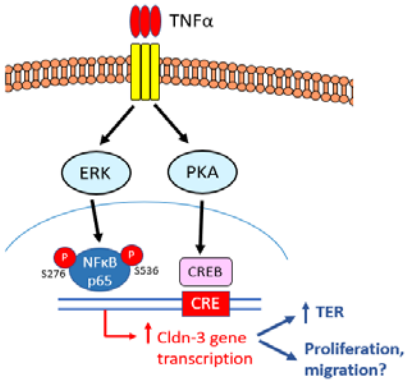
Nanoparticles have gained traction as vehicles for drug delivery because they can overcome limitations associated with free drugs. Nanoparticles are especially advantageous for treatment of cancers, as they can be designed to selectively accumulate in diseased tissues and thus overcome the typical systemic effects associated with chemotherapies. When nanoparticles are injected into biological mediums such as blood, they get coated with proteins and other biomolecules. This protein "coat" changes the characteristics of nanoparticles, affording them a new identity that ultimately affects their fate in biological systems. To ensure nanoparticles can be used to their full potential, a basic understanding of protein-nanoparticle interactions needs to be established. This research aims to answer the question: What are the kinetics and thermodynamics between bovine serum albumin and fluorescent polystyrene nanoparticles, and how do changes in size and temperature affect these parameters?

This research will assess the interactions between fluorescent polystyrene nanoparticles, or fluorospheres (FS) and bovine serum albumin using two-photon excitation fluorescence cross-correlation spectroscopy (TPE-FCCS). This technique is advantageous over other techniques in the field because it allows for direct, in situ measurement of fluorescently-labeled species and their binding interactions. TPE-FCCS will be used to measure the kinetic and thermodynamic parameters of albumin-nanoparticle interactions and to gain an understanding of the forces that dictate protein coating. Thus far, the results suggest low binding ratios (~18 proteins/sphere) and on-rates on the order of 10-4 s-1 for fluorospheres of 100nm size. These findings challenge the current understanding of the formation of a protein monolayer, or "protein corona". The long-term goal of this research is to study more complex protein-nanoparticle interactions, which will allow us to predict the fate of nanoparticles in vivo. By understanding these interactions, we can tailor nanoparticles to be more effective for drug delivery and imaging of diseased tissue.

SHAISTA ANWER: REGULATION AND ROLE OF THE TIGHT JUNCTION MOLECULE CLAUDIN-3 IN EPITHELIAL CELLS

Supervisor: **Dr. Katalin Szaszi**

Epithelial tight junctions (TJ) are essential structures that maintain apico-basal polarity, regulate paracellular permeability and serve as signaling hubs. The claudin family of TJ membrane proteins were identified as the mediators of paracellular transport. In addition, recently several members of the claudin family were also found to modulate signaling pathways, leading to an effect on proliferation, migration and differentiation. The regulation of individual claudin molecules however remains incompletely defined. Here we explored effects of the inflammatory cytokine Tumor Necrosis Factor-α (TNFα) on Claudin-3 (Cldn-3). Cldn-3 is a paracellular sealing protein expressed in the kidney and intestine. Altered expression of Cldn-3 was detected in a variety of cancers and this was associated with metastasis formation and poor prognosis. In this study we showed that TNFα elevated TJ-associated Cldn-3 expression in kidney tubular and colon cancer cell lines. In LLC-PK1 tubular cells TNFα augmented Cldn-3 mRNA but did not affect the turn-over of the protein. Using specific pharmacological inhibitors and siRNA-mediated silencing we showed that Cldn-3 upregulation required extracellular signal regulated kinase (ERK)-dependent activation of the inflammatory transcription factor NFκB. Further, protein kinase A (PKA)-induced CREB activation was also indispensable for the effects, although CREB activation by itself was not sufficient to elevate Cldn-3 protein levels. Finally, we showed that overexpression of Cldn-3 protein elevated transepithelial resistance (TER). Conversely, siRNA-mediated silencing of Cldn-3 inhibited recovery of the epithelial layer in a wound healing assay. Thus, Cldn-3 is necessary for optimal epithelial migration. Ongoing studies are aimed at exploring the mechanism of this effect. Taken together, inflammation-induced upregulation of claudin-3 may significantly alter epithelial permeability and modulate repair of the injured epithelial layer.



SHELLEY VANDERHOUT: COW'S MILK FAT AND CHILD ADIPOSITY: A PROSPECTIVE COHORT STUDY

Supervisor: **Dr. Jonathon Maguire**

Introduction: Cow's milk is a dietary staple for children. International guidelines suggest children older than 2 years of age consume reduced (0.1-2% milk fat) instead of whole (3.25% milk fat) cow's milk to prevent childhood obesity. The objective of this study was to determine the relationship between cow's milk fat (0.1-3.25%) intake and adiposity, measured by Body Mass Index z-score (zBMI) among children aged 9 months-8 years.

Methodology: A prospective cohort study was conducted. Children 9 months to 8 years of age were followed during health maintenance physician visits through the TARGet Kids! primary care research network. The primary exposure was cow's milk fat consumption measured by parental report using a validated dietary questionnaire. The primary outcome was zBMI. Height and weight were measured by trained research assistants and zBMI was determined according to the WHO growth standards. Linear mixed models, adjusted for clinically relevant covariates, were used to determine the longitudinal association between cow's milk fat intake and child zBMI.

Results: Among children aged 9 months to 8 years of age (N= 7467) included in the analysis, each 1% increase in cow's milk fat consumed was associated with a 0.05 lower zBMI score (95% CI -0.06 to -0.03, p< 0.0001). Compared to children who consumed reduced fat (0.1-2%) cow's milk, children who consumed whole cow's milk had 0.82 (95% CI 0.69 to 0.97, p= 0.02) the odds of overweight or obesity.

Conclusions: Guidelines which recommend children to consume reduced fat instead of whole cow's milk may not be effective in preventing child overweight or obesity. Randomized clinical trial evidence is needed to evaluate whether the observed relationship between cow milk fat and child adiposity is causal.

STEPHANIE NISHI: NUT CONSUMPTION DOES NOT INCREASE RISK OF ADIPOSITY: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

Supervisor: **Dr. John Sievenpiper**

Introduction: Nuts have been shown to have cardiovascular and diabetes related health benefits, yet there remains concern that nuts may contribute to weight gain due to their high energy density. Our objective was to conduct a systematic review and meta-analysis of the effect of nut consumption on markers of adiposity in randomized controlled trials using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach.

Methods: MEDLINE, EMBASE, and Cochrane databases were searched (through January 4, 2019). Randomized controlled trials ≥ 3-weeks assessing the effect of nut intake on measures of adiposity were included. Three independent reviewers extracted relevant data and assessed risk of bias of included trials. Data were pooled using the generic inverse variance method and expressed as mean differences (MDs) with 95% confidence intervals (CIs). Heterogeneity was assessed (Cochran Q statistic) and quantified (I2 statistic). The overall certainty of the evidence was assessed using GRADE approach.

Results: 79 randomized controlled trial comparisons involving 4453 people met eligibility criteria. There was no effect of nut consumption on global adiposity (BMI: MD -0.18 [95% CI:-0.41, 0.05]; body weight: MD 0.10 [95% CI: -0.18, 0.39], % body fat: MD -0.26 [95% CI: -0.64, 0.12]) or abdominal adiposity (waist circumference: MD -0.58[95% CI: -1.11, -0.05]). The overall certainty of the evidence was graded as "moderate" for all outcomes owing to inconsistency.

Conclusions: Pooled analyses show nut consumption does not have an adverse effect on measures of adiposity. The concern that nuts may result in weight gain owing to their high energy density appears unwarranted.

Protocol registration: ClinicalTrials.gov Identifier, NCT02654535

Funding: PSI Foundation, Canadian Institutes of Health Research, Canadian Diabetes Association, Banting and Best Diabetes Centre, Ontario Graduate Scholarship, Peterborough

KM Hunter Charitable Foundation Graduate Award.

THENUKA THANABALASINGAM: RECONSTRUCTION OF THE LUNG WITH TYPE II ALVEOLAR EPITHELIAL CELLS ON GELFOAM SPONGE IN EMPHYSEMA

Supervisor: **Dr. Haibo Zhang**

Introduction & Objectives: Pulmonary emphysema, defined as the permanent enlargement of distal air spaces accompanied by the destruction of alveolar walls, leads to a progressive impairment of lung function. Restoring the damaged alveolar architecture by tissue engineering may ameliorate the disease. The hemostatic Gelfoam sponge could be a suitable scaffold for this purpose as the porous structure of the sponge mimics alveolar architecture. On the Gelfoam, we propose culturing type II alveolar epithelial cells (AECII), cells recognized as the alveolar stem cells capable of regenerating the alveolar epithelium. We hypothesize that implantation of AECII-loaded Gelfoam sponges into emphysemic lungs will aid in restoring the alveolar architecture and pulmonary function.

Methods: 50 IU of elastase was administered intratracheally to male Sprague-Dawley rats to induce emphysema. Twenty-eight days later, the Gelfoam sponge was surgically implanted into the left lung. In parallel, induced pluripotent stem cells were differentiated into lung bud organoids (LBOs), which contain pulmonary endoderm and, thus, the potential to generate AECIIs. Then, cells were seeded onto Gelfoam sponge and cultured to promote AECII differentiation. Following culture on the sponge, markers for AECII phenotype were detected by immunocytochemistry (ICC).

Results: Establishment of emphysema was confirmed by histology and decreased lung elastance. Emphysemic rats tolerated surgical implantation of the Gelfoam sponge. ICC analysis of LBO cells cultured on the Gelfoam in vitro revealed expression of AECII markers, EpCAM and pro-surfactant protein C, indicating that the sponge supports differentiation of LBOs to AECIIs.

Conclusion & Future Directions: The in vitro culture of AECIIs on the Gelfoam sponge and the in vivo delivery of the sponge to emphysemic rats points to the feasibility of using the Gelfoam sponge as a scaffold and delivery vehicle of AECIIs to the injured lungs, which could lead to the alveolar regeneration in emphysema.

TIANZHOU ZHANG: A CRITICAL NUAK1-YAP/TAZ FEED FORWARD LOOP DRIVES KIDNEY FIBROSIS

Supervisor: **Dr. Darren Yuen**

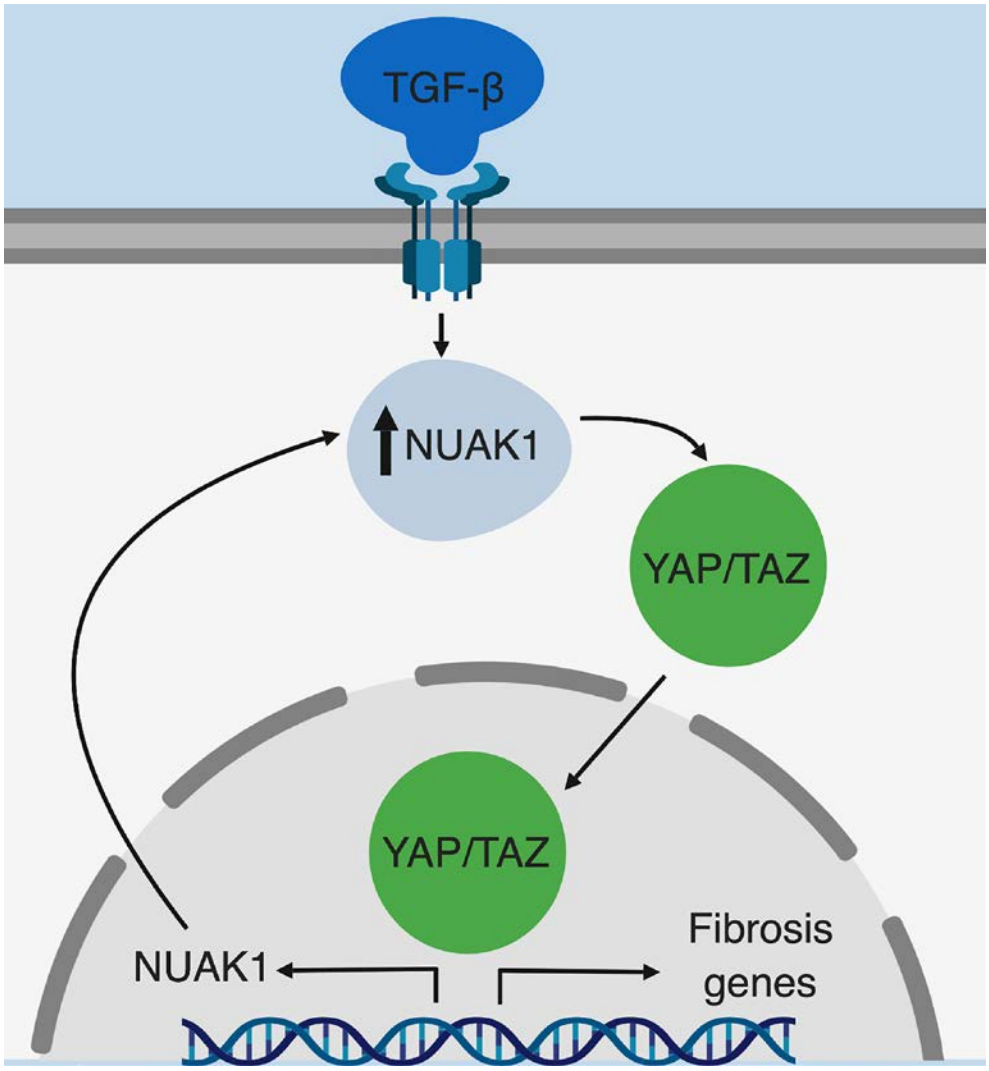
Background: Fibrosis represents a final common damage pathway that is activated by nearly all forms of chronic kidney injury. Recent work from our lab and others has shown the transcription co-factors YAP and TAZ, is a critical inhibitor of fibrosis. NUAK1 is an AMPK-related kinase that we recently found expressed by kidney fibroblasts.

Objectives: To test whether NUAK1 is a driver of kidney fibrosis.

Methods: The role of NUAK1 in the regulation of Hippo pathway activity and fibroblast activation was studied both genetically (silencing) or pharmacologically (using inhibitor) in NRK49F rat kidney fibroblasts. In vivo, we generated tamoxifen-inducible fibroblast-specific NUAK1 knockout mice and subjected them to unilateral ureteral obstruction and folic acid nephropathy. Similarly, we tested the effects of NUAK1 inhibition as an anti-fibrotic treatment strategy. Finally, we profiled human kidney biopsies using RNA-seq to test whether NUAK1 expression correlated with fibrosis and renal outcome.

Results: Both deletion and inhibition of NUAK1 in vitro resulted in increased Hippo pathway activity, as evidenced by increased YAP/TAZ phosphorylation, nuclear YAP/TAZ exclusion, and reduced expression of YAP/TAZ-inducible genes. NUAK1 inhibition also blocked basal and TGF-β-induced fibroblast activation and expression of extracellular matrix genes. Interestingly, YAP/YAZ induced NUAK1 expression, suggesting the existence of a pro-fibrotic NUAK1-YAP/TAZ positive feedback loop. In vivo, fibroblast-specific NUAK1 deletion reduced YAP/TAZ activation and prevented both UUO- and folic acid-induced fibrosis. Similarly, NUAK1 blockade inhibited YAP/TAZ activation and fibrosis immediately after injury, but also when started after fibrosis was already established. Finally, renal NUAK1 mRNA levels correlated strongly with fibrosis and loss of renal function in a cohort of human kidney transplant recipients.

Conclusion: NUAK1 is an unrecognized driver of kidney fibrosis, working at least in part through regulation of the Hippo pathway. Our studies point to the potential for targeting NUAK1 as a novel renoprotective and anti-fibrotic treatment strategy.



Summary schematic describing the proposed mechanism of action of fibroblast NUAK1 as a driver of fibrosis. TGF-b stimulates a rapid increase in NUAK1 protein that leads to activation of YAP/TAZ, as evidenced by reduced YAP phosphorylation, increased nuclear YAP/TAZ localization, and increased transcription of YAP/TAZ-inducible genes. This NUAK1-mediated YAP/TAZ activation drives the expression of fibrosis-associated genes. One of the other genes induced by YAP/TAZ is NUAK1, creating a pro-fibrotic positive feedback YAP/TAZNUAK1 signaling loop that drives ongoing fibrosis. Illustration made in ÓBiorender –biorender.com.

TIFFANY NI: TWO BIRDS ONE STONE: SALVIANOLIC ACID B INHIBITS COAGULATION AND PLATELET AGGREGATION

Supervisor: **Dr. Heyu Ni**

Introduction: Danshen (salvia miltiorrhiza root extracts) have been reported for centuries to control cardiovascular diseases in Chinese medicine. Danshen depside salts are currently approved in China to treat coronary heart disease and angina; and are under evaluation in phase II clinical trials. Salvianolic acids have been identified as the active compounds of danshen and salvianolic acid B (SAB), the most abundant salvianolic acid, has been previously shown to exhibit anti-platelet and anti-thrombotic properties in animal models. However, the mechanism of action has not been adequately explored.

Results: We demonstrate that SAB attenuates ADP-, collagen-, and thrombin receptor activating peptide (TRAP)-induced human platelet aggregation. Interestingly, SAB inhibits thrombin-induced platelet aggregation far more potently than other agonists. Furthermore, using our intravital microscopy thrombosis models, we demonstrate that SAB decreases thrombus growth in vivo. Using a series of in vitro coagulation assays, we found that SAB significantly reduced clot weight in human whole blood, and delayed coagulation in human cell free plasma using thromboelastography. In addition, SAB reduces fibrin network density in cell-free plasma. Through structural analysis, we found that SAB contains structural similarities to trisubstituted benzimidazole thrombin inhibitors, such as dabigatran. In silico molecular modeling predicts that SAB binds within the thrombin active site - interacting with similar residues as dabigatran. Using isothermal titration calorimetry and kinetic thrombin inhibition assays, we corroborate these findings and report SAB as a direct competitive thrombin inhibitor.

Conclusions: These data establish a novel mechanism of SAB in the inhibition of both platelet aggregation and blood coagulation. These unique characteristics position salvianolic acids as safe and potent herb-derived anti-thrombotic agents.