DREAM 2022 SYMPOSIUM
Diabetes Research Envisioned and Accomplished in Manitoba

Making a difference in type 1 diabetes; bench to bedside and back

11th Annual DREAM symposium

Thursday December 1, 2022

Pediatric Grand Rounds

8:00-9:00	**9th Annual Dr. Heather Dean Lecture for Excellence in Pediatric Diabetes Research** ***Dr. Korey Hood,*** *Stanford University* Optimizing Access to and Use of Diabetes Devices
9:25-10:25	***Dr. Carmella Evans-Molina****, Indiana University* The Ailing and Diabetic β Cell in Type 1 Diabetes: Insights From a Trip to the ER
10:25-10:55	***Cameron Keighron - patient partner*** How can we meaningfully include PPI in our research?
11:15-11:45	***Dana Greenberg - patient partner*** How to engage people with lived experience in the research process
12:30-1:30	***Dr. Jennifer Yamamoto****, University of Manitoba* Technology in the Management of Diabetes in Pregnancy: the past, present and future
1:30-2:15	***Dr. Jon McGavock and Dana Greenberg*** Designing clinical trials with patient partners
2:30-3:30	***Dr. Jane Yardley****, University of Alberta* Improving Precision for Exercise Advice in Type 1 Diabetes

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The Children's Hospital Research Institute of Manitoba

greetings

from the CHRIM scientific director

Terry Klassen
CEO and Scientific Director, CHRIM

Welcome to the 11th annual DREAM research symposium!

The DREAM symposium has become a platform to highlight recent discoveries by DREAM researchers and their trainees that lead to improvement of the health of children living with, or at risk for diabetes. In addition, the symposium provides an opportunity to hear from some of the world's best and brightest stars in diabetes and health research. The symposium will begin with the 9th Annual *Heather Dean Lecture in Excellence in Diabetes* by Dr. Korey Hood from Stanford University. On behalf of CHRIM I genuinely invite you to enjoy the day and acquaint yourself in these and other exciting new areas of diabetes research.

from the DREAM co-leads

Allison Dart and Vern Dolinsky
Co-leads, DREAM

Welcome to the 11th annual Diabetes Research Envisioned and Accomplished in Manitoba (DREAM) research symposium. 2022 was a successful year for the research team as we brought together new researchers to the team, obtained a new team grant to study why children born to parents with type 2 diabetes are at higher risk of diabetes and kidney disease and welcome a new Co-lead, Dr. Allison Dart to the team, bringing fresh ideas as we chart future research directions.

We are pleased to once again host an outstanding group of international and national speakers that span the continuum of research from basic discoveries to clinical investigation, with a theme focused on type 1 diabetes in youth. We thank our sponsors, the Children's Hospital Research Institute of Manitoba (CHRIM) and Eli-Lilly for providing funding to make this meeting possible. We are pleased to partner with the Department of Pediatrics on the 9th annual Dr. Heather Dean Lecture in Excellence in Diabetes Research. This year's speaker, Dr. Korey Hood will be presenting on the access and use of diabetes devices. We hope that you will enjoy this symposium!

Dr. Heather Dean Lecture

in Excellence in Diabetes

The 11th Annual DREAM Symposium marks the 9th Annual Dr. Heather Dean Lecture in Excellence in Diabetes. This lecture was named in honour of one of the University of Manitoba's most recognized and trailblazing clinician scientists. Dr. Dean has been a pillar in several communities in our beloved province for nearly 40 years, including but not limited to the medical community, the pediatrics and child health community, the farming community, the sporting community and most famously, the knitting community. Dr. Dean has inspired countless trainees, patients, families and athletes during her tenure in the province. The DREAM team thought it was important to name the opening lecture for our symposium in Dr. Dean's name as without her dedicated commitment to diabetes in children and vision for team-based care, the DREAM team would not exist. The Annual Dr. Heather Dean Lecture in Excellence in Diabetes will symbolize the excellence in clinical care, research and interdisciplinary collaboration in the area of pediatric endocrinology that Dr. Dean has embodied and cultivated in the province of Manitoba. We hope that the lecture will also serve as an annual source of inspiration for young hearts and minds in the same way that Dr. Dean has inspired us over the past 30 years.

Dr. Korey Hood

Korey Hood, PhD
Professor & Staff Psychologist
Stanford University

"Optimizing Access to and Use of Diabetes Devices"

The objectives of this presentation are to share extensive data on gaps and disparities in access to diabetes devices, provide a framework for addressing these gaps, and show tools, in person and digital, to optimize access and use.

Biography:
Korey Hood has been deeply involved in diabetes research, practice, and advocacy for 25 years. The two areas of focus in Dr. Hood's work are to improve how to recognize and deal with psychosocial factors in people with diabetes and ways to utilize diabetes devices to improve health and psychosocial outcomes. Dr. Hood's team has published over 200 scientific articles and created DiabetesWise, a free online platform for people with diabetes and those providing care to them.

keynote speakers

Dr. Carmella Evans-Molina

"The Ailing and Diabetic β Cell in Type 1 Diabetes: Insights From a Trip to the ER"

Carmella Evans-Molina
Indiana University

Dr. Carmella Evans-Molina is the Eli Lilly Professor of Pediatric Diabetes at the Indiana University School of Medicine in Indianapolis, IN, where she serves as Director of the IU Center for Diabetes and Metabolic Diseases and as a Staff Physician at the Roudebush VA Medical Center. She is a Co-Executive Director of the Network for Pancreatic Organ Donors with Diabetes (nPOD) and is a Co-PI of the NIH Integrated Islet Distribution Program. Dr. Evans-Molina's basic science research is focused on understanding how impaired calcium handling in the secretory pathway contributes to β cell dysfunction in diabetes. In addition, she has a clinical research interest focused on defining the natural history of β cell loss in type 1 diabetes and the use of "omics" approaches to identify novel serum biomarkers of β cell stress in pre-symptomatic diabetes. She is an investigator in the NIH-funded Type 1 Diabetes TrialNet, RADIANT, and TIDAPC Networks.

Dr. Jane Yardley

"Improving Precision for Exercise Advice in Type 1 Diabetes"

Jane Yardley
University of Alberta

Jane Yardley, PhD, is an Associate Professor of Physical Education at the University of Alberta's Augustana Faculty in Camrose, Alberta, and a member of the Alberta Diabetes Institute. She is a co-author of the 2016 American Diabetes Association Consensus Statement on Exercise and Physical Activity in Diabetes, and a recipient of the Heart and Stroke Foundation of Canada, Alberta New Investigator Award. Jane's earlier work focused on blood glucose responses to resistance exercise, and the impact of exercise in fasted state. Her recent work has focused on sex and gender-related differences in exercise behaviours and blood glucose responses to exercise in people with type 1 diabetes. She attributes most of what she knows about collaboration and teamwork in research from her transformative experience as a postdoctoral fellow under the supervision of Dr. Jon McGavock.

keynote speakers

Dr. Jennifer Yamamoto

"Technology in the Management of Diabetes in Pregnancy: the past, present and future"

Jennifer Yamamoto
University of Manitoba

Jennifer Yamamoto's research and clinical interest centre around diabetes in pregnancy. Specifically, her research program is interested in how we can leverage diabetes technology to improve outcomes in pregnancies complicated by type 1, type 2, and gestational diabetes.

Dr. Jon McGavock

"Designing clinical trials with patient partners"

Jon McGavock
University of Manitoba

Jon McGavock's research program has 3 main pillars in order to span the spectrum of mechanistic human physiology studies to community-based participatory action research. Pillar 1: the determinants of type 2 diabetes and the associated complications, Pillar 2: dose of physical activity needed to prevent type 2 diabetes and improve metabolic control in individuals with type 1 diabetes, Pillar 3: the role of peer mentoring for the prevention and treatment of type 2 diabetes in youth.

Cameron Keighron

"How can we meaningfully include PPI in our research?"

Cameron Keighron

Cameron has been living with Type 1 diabetes for 12 years and became involved with the D1 Now study when it began in 2014. D1 Now focuses on improving healthcare outcomes for young people with Type 1 diabetes, from the perspective of young people with diabetes. Cameron is currently completing their PhD in Stem Cell therapeutics for Parkinson's Disease at the University of Galway. They are passionate about including the voices of people with diabetes in research, ensuring that the focuses and directions of research come from those living with the disease. This presentation will focus on how we developed a Patient Public Involvement (PPI) model within our own research and some reflections on how best to include PPI in diabetes research.

keynote speakers

Dana Greenberg

"How to engage people with lived experience in the research process"

Dana Greenberg

Dana Greenberg was diagnosed with Type 1 Diabetes (T1D) in 1972, at the age of 7. She was a professional fundraiser for over 20 years and is the proud mom of 3 kids, aged 28, 26 and 20. Twelve years ago, her youngest daughter Marley was also diagnosed with T1D at the age of 8. After Marley's diagnosis, Dana quickly came to realize that she was in a unique position to help others because she understood both what it means to be a person living with T1D and what it means to be a parent of a child with T1D.

Dana is an active volunteer with many diabetes organizations in Toronto, where she chairs various committees, runs speaking engagements, and has mentored dozens of families living with T1D. Dana has been involved with Diabetes Action Canada as a patient partner since 2017. Dana is a member of the Collective Patient Circle, is a patient partner on several research projects, and co-leads the project: *Answering Questions that Matter to Persons Living with Diabetes Using the National Diabetes Repository*.

abstract 1

Replicative Senescence in Pancreatic Beta Cells Increases Extracellular Vesicle Release

Benjamin Bydak, Marvin Yan, Patience O. Obi, Tamiris F. G. Souza, Carmen Ching, Adrian Kee Kong Teo, and Ayesha Saleem

Introduction: Pancreatic β-cell senescence has been shown to contribute to the pathogenesis of type 2 diabetes. Senescent cells secrete higher amounts of pro-inflammatory cytokines as well as extracellular vesicles (EVs). EVs are membrane-bound nanoparticles that are critical in cellular communication and can exert autocrine, paracrine and endocrine effects. EVs vary in size: small-EVs (sEVs) *vs.* medium/large-EVs (m/lEVs) and enclosed cargo. Little is known about EVs released from pancreatic β-cells during replicative senescence.

Methods: Low-passage (LP) and high-passage (HP) murine pancreatic β-cells (MIN6) were grown in EV-depleted media for 48hrs. EVs were isolated from LP (P22-30) and HP (P50-60) conditioned media using differential ultracentrifugation and ultrafiltration, and characterized using tunable resistive pulse sensing (N=6). Cell viability was determined (trypan blue exclusion), cells harvested, and lysates frozen at -80°C for future analysis of senescence markers.

Results: sEV concentration was ~23-fold higher in LP-cells (1.18E+09 particles/ml; p=0.0002) and ~16-fold higher in HP-cells (1.35E+0.9 particles/ml; p<0.0001) *vs.* m/lEVs in each group, illustrating a preponderance of sEV release from cells irrespective of passage. Comparing between passages, secretion of m/lEVs was 1.77-fold higher in HP-EVs *vs.* LP-EVs (p=0.02, N=6). No significant increase was observed in sEV secretion from HP *vs.* LP cells. Average EV size was 9% lower in HP-EVs (113nm) *vs.* LP-EVs (125nm; p=0.04, N=5). EV protein yield, cell count and viability remained unchanged across groups.

Conclusion: Our data show: 1) a preferential release of small-EVs from MIN6 cells irrespective of passage, 2) HP-EVs are smaller in average size, and 3) a 1.77-fold increase in m/lEVs secretion in HP-MIN6 cells. Overall, there is increased EV release with replicative senescence in MIN6 cells. The upstream pathways regulating EV biogenesis, and the functional effects of senescent cell-derived EVs have yet to be elucidated.

abstract 2

The Stability of MIF Receptor CD74 Contributes to the Development of NAFLD

Liujun Chen, Yiheng Huang, Lisha Li, Yadan Qi, and Dake Qi

Introduction: Non-alcoholic fatty liver disease (NAFLD) is associated with metabolic dysfunctions, such as obesity and diabetes. In patients with type 1 diabetes, the prevalence of NAFLD-related liver fibrosis was 16-21%. Macrophage migration inhibitory factor (MIF) is a pro-cytokine which is typically involved in innate immune response. More recently, MIF was also identified to regulate NAFLD, but the molecular mechanisms are largely unknown. Our present study for the first time indicates that MIF provides a non-inflammatory effect on triggering NAFLD through stabilizing its cell membrane receptor, CD74.

Methods: This project combined cell culture, *in vivo* animal models and incorporating molecular & cellular techniques to explore a cellular mechanism of MIF/CD74/NAFLD.

Results: MIF treatment increased CD74 protein contents rather than gene expression in HepG2 cells in a time-dependent manner. The alteration of CD74 was not associated with any changes in the expression of proinflammatory factors, such as TNFa, IL-6 and IL-1b. In a MIF overexpressed animal model (Mif lung Tg), high circulating MIF levels were also associated with increased hepatic CD74 proteins but not genes in the absence of inflammation in the liver. High fat diet increased levels of circulating MIF leading to an upregulation of CD74, steatosis and fibrosis in the liver. These alterations could be reversed by either MIF neutralization or MIF knockout, suggesting a key role of MIF in stabilizing hepatic CD74 and regulating the development of NAFLD. The stability of CD74 is related to MIF-mediated functions of caspase 4 but not through binding with MIF.

Conclusion: MIF has a post-transcriptional effect on stabilizing CD74 that regulates the development of NAFLD.

abstract 3

The Role of Nix in Muscle Metabolism: A Mitophagy Receptor Regulates Metabolism and Insulin Sensitivity

Jared T. Field, Donald C. Chapman, Richard LeDuc, Jason Kindrachuk, Ayesha Saleem, Barbara Triggs-Raine, and Joseph W. Gordon

Introduction: Early in type 2 diabetes, skeletal muscle ceases to respond to insulin signaling. Over-activation of mitochondrial quality control pathways contribute to muscle insulin resistance. The protein Nix is implicated to be central in muscle metabolism and has been demonstrated to regulate insulin signaling, mitochondrial turnover, and growth and development in muscle. We generated a mouse model where Nix was specifically deleted in muscle to test the hypothesis: Ablation Nix in muscle improves insulin signalling through regulation of mitochondrial clearance and mitochondrial turnover.

Methods: To determine the effect of Nix, muscle-specific deletion of Nix in mice was achieved using Cre-lox recombination and used in a series of histological, biochemical, and physiological tests. To assess cellular mechanisms, a cell culture model of C2C12 myotubes was used with fluorescent microscopy.

Results: Deletion of Nix in muscle caused the appearance of ragged red fibers, a marker of accumulated senescent mitochondria, which was confirmed by electron microscopy. In the cellular model, we observed that depleting Nix levels resulted in impaired mitochondrial clearance which supports the phenotypes observed *in vivo*. Array-based analysis of kinase activity (kinomics) suggested that Nix-deletion mice has reduced insulin-inhibitory signaling (mTOR/S6K) but enhanced storage of lipid and glycogen. Histological staining similarly indicates enhanced glycogen deposition in the muscle without Nix. Lastly, physiological tests showed that Nix-knockout mice had improved insulin sensitivity but reduced basal metabolic rate and decreased exercise endurance.

Main Findings: Together these data provide evidence that deletion of Nix results in impaired mitochondrial clearance, decreased exercise tolerance, but also had improved Sensitivity to insulin and storage of fuels. In conclusion, Nix is a potential therapeutic target in diabetes due to roles in regulating muscle mitochondria turnover and insulin signaling which are key pathways for to the development of insulin resistance in muscle and type 2 diabetes.

abstract 4

The Role of Pref-1 in Non-inflammatory Insulin Resistance

Yiheng Huang, Liujun Chen, Yadan Qi, Lawrence H. Young, Richard Bucala, and Dake Qi

Introduction: Insulin resistance (IR) in obesity is considered to arise from inflammation in white adipose tissue (WAT). However, anti-inflammatory therapies have failed to improve insulin sensitivity in either animal models or human subjects. Recent studies further indicate that obesity-induced IR may occur without any change in systemic or tissue inflammation. IR can develop before macrophage accumulation and WAT inflammation suggesting a proximate role of non-inflammatory adipose events in the initiation of IR. However, the underlying molecular and cellular mechanisms responsible for these observations remain largely unknown. We report a non-inflammatory adipose mechanism of IR mediated by loss of Pref-1.

Methods: This project combined human study, cell culture and *in vivo* animal models, and incorporating molecular and cellular techniques, flow cytometry and *in vivo* transplantation.

Results: Pref-1, released from adipose Pref-1+ cells with characteristics of M2 macrophages, endothelial cells or progenitors, inhibits MIF release from both Pref-1+ cells and adipocytes by binding with integrin $\beta 1$ and inhibiting the mobilization of p115. High palmitic acid (PA) induces PAR2 expression in Pref-1+ cells, leading to the downregulation of Pref-1 expression and release in an AMPK-dependent manner. The loss of Pref-1 increases adipose non-inflammatory MIF secretion contributing to IR in obesity. Treatment with Pref-1 blunts the increase in circulating plasma MIF levels and subsequent IR induced by a high PA diet.

Conclusion: High levels of fatty acids induce a loss of Pref-1 expression and secretion, through increased activation of PAR2, which leads to an increase in MIF secretion and a non-inflammatory adipose mechanism of IR.

abstract 5

Development of a Mouse Model of Type 2 Diabetes (T2D) During Pregnancy to Test how *In Utero* Exposure to T2D Impacts Offspring Metabolic Health

Maryana Y. Kutuzova, Kristin L. Hunt, and Christine A. Doucette

Introduction: Recent studies demonstrate that *in utero* exposure to type 2 diabetes (T2D) increases the risk for T2D in offspring; however, the mechanisms of this intergenerational risk transmission are unclear, partly because we lack an appropriate model to induce pre-gestational T2D in mice. Existing rodent models of T2D during pregnancy do not phenocopy human T2D and often use extreme measures to kill the pancreatic beta cells, resulting in atypical severe hyperglycemia, which itself can influence the metabolic health of the offspring. An appropriate model of T2D during pregnancy that more accurately phenocopies T2D in humans is needed.

Methods: To determine the best conditions to induce T2D in female mice, we exposed C57BL6 mice to a high fat and sucrose (HFS; 4, 6 or 10 weeks) diet to induce insulin resistance. Further to this, a one-time administration of streptozotocin (STZ; 75 mg/kg, 100 mg/kg, or 150 mg/kg) was used to kill some beta cells and impair insulin secretion, but not eliminate the beta cell pool completely. Weekly body weight and biweekly fasting blood glucose measurements. Two weeks after STZ injection, mice underwent a glucose tolerance test and insulin tolerance test (ITT) to demonstrate the degree of T2D development.

Results: Our preliminary results suggest that the mice that consumed a HFS diet for 6 weeks followed by a 150mg/kg STZ injection developed glucose intolerance with mild suppression of plasma insulin levels during the GTT.

Conclusion: While further characterization of this model is needed, our preliminary findings suggest that through a combination of HFS diet and STZ we can induce T2D in female C57BL6 mice. Future studies will confirm these findings and characterize the impact of this exposure on the metabolic health of the offspring.

abstract 6

The Attenuation of Cardiac DDT Expression Associated with Metabolic Dysfunction Exacerbates Myocardial Ischemia-Reperfusion Injury

Lisha Li, Yiheng Huang, Liujun Chen, Yadan Qi, Lawrence Young, Richard Bucala, and Dake Qi

Introduction: Metabolic abnormalities including obesity, insulin resistance, hyperlipidemia and diabetes, affect clinical recovery and long-term survival in acute myocardial infarcted patients receiving reperfusion therapy. However, to date the mechanisms by which metabolic dysfunction exacerbates cardiac ischemia-reperfusion injury have yet to be understood. D-dopachrome tautomerase (DDT) is a homolog of the pro-inflammatory factor macrophage migration inhibitory factor (MIF). Its expression and release in cardiomyocytes protect the heart from injury during hypoxia-ischemia in mice. DDT knockout in cardiomyocytes is associated with exacerbated cardiac injury compared to WT hearts following ischemia-reperfusion. This study investigated the regulation of DDT in cardiomyocytes by metabolic dysfunction and ***innovate DDT as a novel therapy*** to limit reperfusion injury in the subjects with metabolic abnormalities.

Methods: This project used a combination of cardiomyocyte cell line, isolated adult cardiomyocytes, *ex vivo* heart perfusion in mice (3 weeks) with or without high caloric diet feeding for 12 weeks.

Results: We found that high caloric diet feeding decreased DDT rather than MIF gene and protein expression in the heart, which leads to augmented cardiac injury in ischemia-reperfusion. Of all the major fatty acids in high caloric diet, palmitic acid has been identified to downregulate DDT expression in cardiomyocytes. The reduction of DDT is associated with upregulated FOXO1 gene expression and nuclear accumulation. The knockdown of FOXO1 in cardiomyocytes reversed PA induced reduction of DDT, suggesting a key role of FOXO1 in downregulating DDT.

Conclusion: High fat diet, and in particular palmitic acid, suppresses cardiac DDT expression through a FOXO1 signaling pathway, which deteriorates ischemia-reperfusion injury and affects clinical outcomes of myocardial infarction.

abstract 7

Mental Health and Progression of Early Kidney Injury in Youth with Type 2 Diabetes: a 2-Year Analysis from the iCARE Cohort

Anna Liu, Melissa Del Vecchio, Elizabeth Sellers, Jonathon McGavock, Brenden Dufault, Brandy Wicklow, and Allison Dart

Background: Psychological distress is common in youth with type 2 diabetes (T2D) and may impact disease management. We hypothesize that youth with more positive mental health will have less progression of early kidney injury.

Aim: To determine if better baseline mental health and resilience protect from progression (or is associated with regression) of albuminuria in youth with T2D.

Methods: We included youth in the iCARE cohort with complete albumin-to-creatinine ratio (ACR) and mental health covariates at baseline and 2-year follow-up. Mental health was assessed using the Kessler Psychological Distress Scale (K6), Perceived Stress Scale 14 (PSS-14), Resilience Scale for Children and Adolescents (RSCA), and Child and Youth Resilience Measure (CYRM). Main outcome was albuminuria, evaluated as normal, progression, regression, or persistent based on KDIGO criteria at 2-year follow-up. A multivariate regression analysis was used to evaluate associations between mental health scores and log relative change in albuminuria (2 years/baseline).

Results: Of the 191 participants included, 68.1% were female, mean age was 14.2 years (SD 2.2) and median HbA1C was 8.9% (IQR 7.1-11.3) at baseline. At baseline, 17.2% were distressed, 54.6% stressed, and median CYRM and RSCA scores were 108.5 (IQR 94.5-121.0) and 37.5 (IQR 28.3-46.0) respectively. Median ACR was 0.96mg/mmol (IQR 0.30-3.42) at baseline and 1.30mg/mmol (IQR 0.56-5.40) at 2-year follow-up. At the 2-year mark, 58.6% remained with normal albuminuria, 9.9% regressed, 14.7% persisted, and 16.8% progressed. Linear regression analysis revealed no significant associations between mental health scores and change in albuminuria.

Main findings: High rates of albuminuria at baseline and 2-year follow-up were observed. We also found high rates of stress and distress amongst participants. While mental health and albuminuria were not associated, both variables are expected to impact health in youth with T2D and should be further explored and addressed in research and clinical care.

abstract 8

The Effects of Breastfeeding on Chronic Kidney Disease in Child Exposed to Maternal Type 2 Diabetes in Utero

Alennie C. Lopez, Elizabeth A. C. Sellers, Lorraine McLeod, Kristine Kroeker, Allison B. Dart, and Brandy A. Wicklow

Background: The prevalence of T2DM complicating pregnancy is increasing. T2DM exposure in utero is associated with congenital anomalies of the kidney and urinary tract and development of albuminuria in childhood. Breastfeeding is associated with larger combined kidney volumes and higher eGFRs at school age, and decreased odds of developing microalbuminuria.

Aim: To determine if breastfeeding initiation is protective against the development of kidney dysfunction in children exposed to T2DM in utero

Methods: A Manitoba population based historical prospective cohort study was created to investigate the association between breastfeeding initiation and the development of kidney disease in offspring exposed to maternal T2DM in utero. All pregnancies resulting in a live birth between 1980 and 2018 were included in analysis. Breastfeeding initiation was determined from hospital discharge record. The primary outcome was chronic kidney disease (CKD) classified by physician billing codes and laboratory data. Group differences were compared by t-tests, Mann-Whitney U tests or chi-square tests as appropriate. A binary regression was used to determine the association between breastfeeding and development of CKD. Kaplan-Meier survival analysis will be used to calculate the cumulative incidence rate for kidney disease in the offspring.

Results: Of 628,125 offspring, 7,642 were exposed to T2DM in utero. Of these, 4,804 offspring had breastfeeding initiated in hospital (62%). CKD was identified in 5.1% of the offspring in the breastfed group and 7.54% of the offspring in the non-breastfed group ($X2= 18.47$, $p<0.0001$). The odds of developing CKD were lower in offspring who initiated breast feeding compared to offspring who did not (OR = 0.67, 95% CI [0.55-0.9=80]).

Main Findings: In offspring who were exposed to T2DM in utero, breastfeeding initiation decreased the odds of developing CKD. These results suggest that breastfeeding initiation could be a potential area of focus to address the intergenerational diabetes cycle.

abstract 9

“How We Do It”: a Qualitative Study of Strategies for Adopting an Exercise Routine while Living with Type 1 Diabetes

Andrea MacIntosh, Dana Greenberg, Jane E. Yardley, Nika Klaprat, Cristine Vlcek, Marley Greenberg, Joel Brandt, Natasha Gregoire, Sylvie Dostie, Denis Boutin, Conrad Pow, Mandy Archibald, and Jonathan McGavock

Introduction: For people living with type 1 diabetes (T1D), engaging in physical activity (PA) is compounded by the risks of hypoglycemia and glucose variability. Little information exists on the lived experience of overcoming these barriers. The purpose of this study was to identify successes and challenges related to exercise, and strategies to be active while living with T1D.

Methods: We conducted a patient-led qualitative study consisting of semi-structured interviews with individuals living with T1D, aged 16 years or older. Relationships with existing patient co-researchers and snowball sampling were used to purposely sample individuals who reported being regularly physically active and lived with T1D for at least one year. Interviews were performed virtually, recorded and transcribed. Data were uploaded to NVivo12, and a descriptive thematic analysis was used to generate themes and strategies. Patient co-researchers were involved in all phases of the study.

Results: Twenty-two interviews were conducted with14 self-identified women and 8 self-identified men (ages 19-62, median age 32 years) with either one of the researchers, or a patient co-researcher and research assistant. We identified 5 themes: (1) Structure and Organization “*I can't do spontaneous exercise. For me, I actually need a couple hours of warning minimum..*”; (2) Trial and Error “*Once you put the time and effort into learning, you will have greater success in how to exercise*”; (3) Psychosocial Aspects “*...because it's not just your body, it's your soul, it's your mind that exercise is for...*”(4) Technology; and (5) Education and Peer Support. Strategies to overcome barriers included (1) The use of Technology; (2) Integrating Psychosocial Facilitators; (3) Insulin and Carbohydrate Adjustments; and (4) Planning for Exercise.

Conclusions: We identified strategies for incorporating PA into the lives of people living with T1D that could inform practice recommendations to facilitate behaviour change, or future trials that include PA interventions.

abstract 10

Development of Pharmacological Strategies to Reduce Amyloid Formation and β-Cell Death in Human Islets – Implications in Type 2 Diabetes and Islet Grafts

Mukta Moni, Abhimanyu Gupta, and Lucy Marzban

Background: Type 2 diabetes (T2D) is characterized by progressive loss of β-cell mass and function, leading to hyperglycemia. An important factor contributing to β-cell failure in T2D is islet amyloid formation caused by aggregation of the β-cell hormone, islet amyloid polypeptide (IAPP). Amyloid also forms in transplanted islets in patients with type 1 diabetes (T1D) and contributes to graft failure. Amyloid formation promotes islet inflammation which is associated with elevated proinflammatory cytokine interleukin-1 beta (IL-1β) in islets. We examined three pharmacological approaches IL-1 receptor antagonist (anakinra), IL-1β neutralizing antibody (nAb), glucagon-like peptide-1 receptor agonist (exenatide), to reduce amyloid formation and its β-cell toxicity in human islets.

Methods: Isolated human islets (n=4 cadaveric donors) were cultured free-floating in elevated (11.1 mmol/l) glucose (to form amyloid) with or without treatment by anakinra (10 μg/ml), nAb (1 μg/ml), exenatide (10 nmol/l) or combined anakinra and exenatide, for 7 days. Quantitative immunolabeling was performed on paraffin-embedded islet sections for insulin, TUNEL (apoptosis), and thioflavin S (amyloid).

Results: Treatment of human islets with anakinra, nAb, or exenatide markedly reduced the number of amyloid-positive human islets (non-treated: 12±4%, anakinra(+): 4±0.8%, exenatide(+): 5±1%, nAb(+): 4.7±1.3%, $p<0.05$) and TUNEL-positive β-cells (non-treated: 7.4±0.9%, anakinra(+): 3.9±0.7%, exenatide(+): 3.6±0.5%, nAb(+): 5.4±0.7%, $p<0.05$). Moreover, combined anakinra and exenatide treatment was more effective than treatment with either anakinra or exenatide alone in reducing both amyloid positive islets (2±0.5%, $p<0.05$) and TUNEL-positive β-cells (2.1±0.3%, $p<0.05$).

Conclusions: These studies suggest that [1] treatment of human islets with anakinra, nAb, or exenatide effectively reduces amyloid formation and amyloid-induced β-cell death; and [2] combined anakinra and exenatide treatment has synergistic effects in reducing amyloid formation and its β-cell toxicity. These studies suggest that IL-1 receptor antagonists and GLP-1 agonists may provide effective therapies for protecting human islets from amyloid toxicity in T2D and islet grafts in T1D.

abstract 11

The HNF-1αG319S Variant Shifts β-cell Metabolism Towards Fatty Acid Oxidation: Implications for Type 2 Diabetes and First Nations Youth

Taylor S. Morriseau, Kristin L. Hunt, Vernon W. Dolinsky, Francis Lynn, and Christine A. Doucette

Background: 40% of First Nations youth with type 2 diabetes (T2D) in Manitoba carry a variant in the HNF-1α gene (HNF-1αG319S). The G319S variant is thought to drive pancreatic β-cell dysfunction; however, youth-onset T2D is a relatively recent phenomenon. We hypothesize the G319S variant impairs insulin secretion when exposed to dietary carbohydrate stress but is protective when consuming traditional off-the-land foods that are rich in fat and protein.

Objective: In the context of colonial impacts on traditional food systems, we aim to define how the HNF-1αG319S variant interacts with diet to influence whole-body metabolism and nutrient-induced insulin secretion.

Methods: CRISPR/Cas9 was used to knock-in the G>A.955 substitution into C57/BL6 mice. Mice were weaned onto (1) a high-fat, low-carbohydrate (HFLC) diet reflecting off-the-land foods, or (2) a high-fat, high-carbohydrate (HFHC) diet reflecting present-day dietary patterns. Glucose and pyruvate tolerance were assessed prior to isolation of pancreatic islets to measure glucose-stimulated insulin secretion (GSIS), gene expression, or respiration.

Results: A HFHC diet induced glucose intolerance, fasting hyperinsulinemia, unrestrained endogenous glucose production, and attenuated GSIS in G319S-expressing female mice between 3- and 6-months-of-age, pointing to dysregulated glucose homeostasis. Conversely, the HFLC diet prevented glucose intolerance in G319S-expressing female mice via the suppression of endogenous glucose production. In isolated islets, fatty acid oxidation was increased 2-fold, and gene expression changes supported a metabolic switch toward fat oxidation that normalized insulin secretion.

Conclusion: In support of our hypothesis, a HFHC diet accelerated metabolic dysfunction in G319S-expressing mice. This was most prominent in females, consistent with the female predominance of youth-onset T2D. Conversely, aligning dietary fat with the observed shift in metabolism using a short-term HFLC diet prevented metabolic dysfunction in G319S carriers. These mechanistic and metabolic observations lend support to the previously established role of traditional foods in protecting against diabetes within First Nations communities.

abstract 12

The Effects of RESV Supplementation Gestational Diabetes and Cardiac Hypertrophy in Offspring Fetal Cardiomyocytes

Marcelo Ninalaya, Mateusz M. Tomczyk, Bo Xiang, Gabriel M. Brawerman, Stephanie M. Kereliuk, and Vernon W. Dolinsky.

Introduction: Gestational Diabetes Mellitus (GDM) is a common metabolic condition that is often observed during late pregnancy. Current GDM treatment strategies include diet, exercise and insulin therapy. Pharmacotherapies have the risk of adverse pregnancy outcomes. Previous studies have found Resveratrol (RESV) to be a potentially safer alternative form of treatment.

Objective: We hypothesize that maternal RESV supplementation will mitigate GDM-induced mitochondrial dysfunction in primary cardiomyocytes. We further hypothesize that maternal RESV treatment can improve GDM-induced impairments in cardiac hypertrophy in primary cardiomyocytes.

Methods: Female rats were fed a low fat (Lean) (10% kcal fat) or high fat and sucrose (GDM) (45% kcal fat) diet 6 weeks prior to mating to induce GDM. At mid-pregnancy, after the appearance of glucose intolerance, a subgroup of GDM dams were switched to a diet supplemented with RESV (GDM+RESV) (45% kcal + 4g/kg RESV). To determine the effects of RESV on the cardiovascular system of GDM-offspring, e.20 pups were sacrificed for cardiomyocyte isolation. Mito-stress and glycolysis stress assays were preformed on isolated cardiomyocytes to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) respectively.

Results: Preliminary echocardiography data found that RESV attenuated GDM-induced cardiac hypertrophy in fetal offspring. Cardiomyocytes from GDM-offspring had lower levels of ATP production and maximal respiratory capacity compared to Lean offspring. Initial analysis found that maternal RESV supplementation attenuated mitochondrial efficiency and capacity in response to high-stress conditions in GDM-exposed primary cardiomyocytes.

Conclusion: Initial findings suggest that maternal RESV supplementation attenuated mitochondrial efficiency and capacity as well as cardiac hypertrophy induced by GDM in fetal cardiomyocytes.

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Investigating the Effect of the HNF-1aG319S Variant on Liver and Pancreas Function under Different Physiological States

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Introduction: Genetic testing in Anishininew communities in central Canada led to the discovery of the HNF-1aG319S variant, which is strongly associated with youth-onset type 2 diabetes. Currently, it is unclear how the G319S variant influences beta cell and liver function. Given the metabolic demand associated with traditional lifestyle practices in central Canada, the G319S variant may instead confer an advantage to prolonged fasting. Here, we examine the impact of prolonged fasting in G319S expressing mice compared to control mice.

Methods: CRISPR/Cas9 was used to knock in the G319S variant in C57BL/6 mice, creating male and female wildtype (G/G), heterozygous (G/S), and homozygous (S/S) mice. At 3 months, mice were sacrificed either under ad libitum condition or after 24 hours fasting. Liver tissues were collected to measure gene expression, triglyceride, and glycogen content. Islets were isolated to assess insulin secretion capacity, and for electron micrography (EM) imaging.

Results: A statistically significant reduction in liver triglycerides was observed in G/S ($p=0.0237$) mice compared to G/G. In addition, increased expression of genes involved in ketogenesis was observed, including HMGCR in G/S ($p=0.0140$) and S/S ($P=0.0073$) mice, as well as increased expression of genes involved in gluconeogenesis, including G6PT-1 in S/S mice ($p=0.0290$). Once fasted, a decrease in blood glucose was observed in G/S ($P=<0.0001$), and S/S ($P=0.0385$) mice compared to G/G, and a trend towards increased blood ketones was also seen. EM images showed an increase percentage of immature insulin granules in male S/S ($p=0.0157$) compared to G/G.

Conclusion: Our findings indicate that the G319S variant alters metabolism in the liver toward ketogenesis and gluconeogenesis, and a propensity toward insulin depletion in the islets, which may indicate that the G319S variant provides a metabolic advantage during extended periods of fasting.

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Can Chronic Contractile Activity-Derived Extracellular Vesicles Rescue mitochondrial Dysfunction?

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Background: Mitochondrial dysfunction is implicated in type 2 diabetes. Chronic exercise improves mitochondrial function. Extracellular vesicles (EVs) are important mediators of intercellular communication and are released into circulation with exercise. We have shown that an *in vitro* model of exercise, chronic contractile activity (CCA)-derived muscle-EVs (CCA-EVs) increased mitochondrial biogenesis in healthy C2C12 myoblasts. We do not know if CCA-EVs can evoke similar effects in cells with impaired metabolism.

Methods: C2C12 myoblasts were treated with 0.25mM H_2O_2 (6hrs) alone, or with 1.0mM N-acetylcysteine (NAC, 24hrs pre-incubation, N=6). Metabolic dysfunction in treated cells was confirmed using MitoTracker red and oxygen consumption rate analysis (Agilent XFe24). EVs were isolated from conditioned media of cells (control and H_2O_2) using differential ultracentrifugation, characterized biophysically (Izon®) and by EV-subtype marker expression (TSG101, Flotillin-1, Cytochrome-C). CCA (3h/d, 4d, 14V, C-PACE EM, IonOptix) was used to mimic chronic exercise *in vitro*. CCA-EVs were isolated as before. Untreated control, H_2O_2 and H_2O_2+NAC cells were incubated with control-EVs or CCA-EVs (4d) and cell viability and metabolism were assessed (N=2).

Results: EV concentration (particles/ml) was 1.58-fold higher in H_2O_2-EVs *vs.* control (p=0.21). EV protein yield was 1.90-fold higher in H_2O_2-EVs (p=0.02). Flotillin-1 was reduced by 35.4% (p=0.03), TSG101 by 51.81% (p=0.08), and Cyt-C was undetectable in H_2O_2-EVs. H_2O_2-treatment induced a 47% decrease in cell viability (p=0.0009), and reduced basal and maximal oxygen consumption rates by 91.3% (p=0.0007) and 98.6% (p=0.0010) respectively. Pre-treatment with NAC rescued cell viability by 26.8% (p=0.23) but did not improve mitochondrial function. Preliminary results indicate CCA-EVs rescued cell viability by 2.0-fold and mitochondrial content by 8.3% in H_2O_2-treated cells.

Conclusion: Mitochondrial dysfunction increased EV concentration and protein yield, and decreased Flotillin-1 expression. CCA-EVs treatment appears to rescue both cell viability and impaired metabolism in H_2O_2-treated cells. Further confirmation of data and underlying mechanisms remain to be elucidated.

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Inhibitors of Intracellular Islet Amyloid Formation Protect Beta Cells from Amyloid Toxicity

Rushie Tyagi, Danish Malhotra, and Lucy Marzban

Introduction: Beta-cell failure is a key defect in type 2 diabetes (T2D) that leads to elevated blood glucose. Formation of toxic protein aggregates named amyloid in pancreatic islets contributes to beta-cell dysfunction and death in patients with T2D. Importantly, islet amyloid also forms in human islets during pre-transplant culture and following transplantation in patients with type 1 diabetes (T1D), which may lead to graft failure. Islet amyloid is formed by aggregation of human islet amyloid polypeptide (hIAPP; amylin), a hormone normally produced by beta cells. Small intracellular hIAPP aggregates are the major toxic form of amyloid. We examined the effectiveness of two inhibitors of hIAPP aggregation (developed in our group) on preventing amyloid-induced beta-cell death in genetically modified beta cells.

Methods: Formation of intracellular hIAPP aggregates was induced in INS-1 rat beta cells using an adenoviral gene expression approach. Expression of hIAPP in INS-1 cells was optimized to levels in human islet beta cells. INS-1 cells were treated with two cell permeable inhibitors of hIAPP aggregation (PEP-A1 and PEP-A2) for 48 hours. Following treatment cells were immunolabelled for insulin, TUNEL (detect apoptosis), and A11 (detect hIAPP aggregates).

Results: Transduced INS-1 cells expressing hIAPP formed intracellular hIAPP aggregates which closely correlated with the elevated number of TUNEL-positive (apoptotic) cells (control: 0.5±0.1%; +hIAPP: 4.1±1.0%; $p<0.05$). Treatment with inhibitors of hIAPP aggregation, PEP-A1 or PEP-A2, markedly reduced hIAPP aggregates and apoptotic cells (hIAPP+PEP-A1: 1.0±0.2%, hIAPP+PEP-A2: 1.4±0.2%). Treatment with inhibitors alone did not have any effect on apoptosis in control cells.

Conclusion: The results of this pilot study suggest that both inhibitors effectively reduced intracellular hIAPP aggregation and its beta-cell toxicity in INS-1 cells. The inhibitors of hIAPP aggregation may provide a new strategy to protect islet beta cells from amyloid toxicity in conditions associated with amyloid formation such as T2D and islet grafts.

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Evaluating the Relationship between Protective Psychosocial Factors and Hypertension in an Overweight Cohort of Indigenous Youth; a Cross Sectional Study

Harman Vats, Allison Dart, Brandy Wicklow, Jon McGavock, and Elizabeth Sellers

Introduction: Indigenous communities are disproportionally affected by chronic diseases due to the impacts of colonization. Despite adversity, many children continue to thrive. We hypothesize that supportive psychosocial factors are associated with lower rates hypertension in an at-risk overweight cohort of Indigenous youth.

Methodology: The study includes controls from the iCARE study. Inclusion criteria: age 10 – 25 years, Indigenous ethnicity, and BMI >85th%ile. Primary outcome: hypertension; by 24-hour ABPM or BpTRU device. Metabolic and psychosocial factors were compared between youth with and without hypertension. A univariate linear regression analysis evaluated associations between metabolic and protective factors and mean daytime systolic and diastolic bp as a continuous outcome.

Results: A total of 129 youth with a mean age of 14.4 years and 54.3% female were included (54 with ABPM and 75 with BpTRU data). Mean BMI z-score was 3.28 +/- 0.28. Overall, 26.4% had HTN (38.9% by ABPM; 22.0% by BpTRU). Youth with hypertension had higher markers of metabolic syndrome: HbA1c (5.66 + 0.274% vs. 5.49 + 0.27%; p = 0.005), ALT (19.0 [10.0, 74.0] vs. 26.0 [9.00, 162], p = 0.03), and glomerular hyperfiltration: eGFR (121 [89.2, 179]ml/min/1.73m2, 131 [94.3, 222] ml/min/1.73m2, p = 0.02). Lower systolic blood pressure was associated with lower stress levels (β= 5.19; p=0.27) and higher food security (β = -9.49; p=0.001), and participation in more vigorous-physical activity (β= -0.048; p=0.047) had lower diastolic blood pressure.

Conclusion: The high rates of hypertension in overweight and obese Indigenous youth support screening with gold standard ABPM. The protective psychosocial factors identified inform systemic changes, and community lead prevention efforts. These important findings need to be explored further to support resource allocation and strengthen protective factors that exist within Indigenous communities.