Abstract 1

A novel transgenic inducible GFP extravesicular reporter mouse for isolating pancreatic beta-cell extracellular vesicles during type 1 diabetes pathogenesis

Mystica Amonyi, Jasmine Pipella, Roozbeh Akbari Motlagh and Peter J. Thompson

Introduction: Type 1 diabetes (T1D) is characterized by insulin deficiency as a result of T cell infiltration of islets and destruction of beta (β) cells. Exosomes, a subdivision of extracellular vesicles (EVs) produced by the endosomal membrane pathway, play a role in cellular communication and contain molecular cargo, including nucleic acids and proteins that change with disease development. While β-cell EVs have been studied from isolated islets ex vivo, the roles they play in vivo in T1D is not known. In vivo studies have been hampered by lack of genetic tools to specifically identify, isolate and characterize β cell EVs during T1D pathogenesis.

Methods: To gain insight into the relationship between β cell EVs and their role in the pathogenesis of T1D, we generated a novel transgenic inducible GFP extravesicular reporter (TIGER) in the nonobese diabetic (NOD) mouse strain, bearing a CRE-recombinase dependent CAG promoter and human CD9-TurboGFPloxP/stop/loxP knock-in. This enables the specific labeling, isolating, and characterization of CD9-GFP+ pancreatic β-cell derived exosomes by induction of the reporter selectively in β cells using an adenoviral-associated vector (AAV) Ins1::Cre construct, in the T1D-prone NOD mouse strain.

Objectives: Our aim is to track and characterize on the population of GFP+ exosomes, their membrane proteins, and the range of packaged cargo during T1D. This EV reporter mouse model will provide a novel genetic tool for the in vivo labeling of pancreatic β cell EVs and insight into their molecular components and trafficking to immune cells during the development of T1D.

Abstract 2

Investigating how in utero Type 2 Diabetes exposure affects kidney structure and function in offspring.

Lydia Amooga, Thiago L. Baltus, Kristin L. Hunt, Christine A. Doucette

Introduction: Kidneys are essential for blood filtration and waste removal. The kidneys develop early during the first trimester of gestation. DREAM clinicians have shown that exposure to maternal type 2 diabetes (T2D) increases the offspring's risk for T2D and chronic kidney disease (CKD); however, its unclear how maternal T2D contributes to pathophysiological changes in the kidneys of exposed offspring. We hypothesize that exposure to maternal T2D will impair kidney development such that offspring will have aberrant glomerular and proximal tubular structure and function, lending to susceptibility to CKD.

Methods: T2D was induced in female C57BL6/J mice prior to pregnancy using a combination of diet-induced obesity (8 high-fat and -sucrose feeding) and beta cell insufficiency (through injection of a mild dose of streptozotocin). Control (chow-fed) and T2D females were mated with healthy males and the offspring assessed for various aspects of kidney structure and function.

Results: Early results suggest that T2D-exposed offspring have significantly higher rates of proteinuria and microalbuminuria. Additional analyses soon to be performed include assessment of urinary NGAL and KIM-1 levels (markers of proximal tubule injury) and comprehensive glomerular and proximal tubule structural analyses.

Conclusion: This study will be the first to define how maternal T2D impacts kidney structure and function in T2D-exposed offspring. Early results indicate that kidney function in mouse offspring is negatively affected in the exposed young-adult, providing insight into the pathophysiology of CKD susceptibility in T2D-exposed offspring. Ultimately, this will help us define better intervention windows and more appropriate drug targets for T2D-exposed offspring.

Abstract 3

Medication Adherence in Youth with Type 2 Diabetes

Joanna Ankama*, Sara Schur*, Allison Dart, Elizabeth Sellers, Jonathan Mcgavock, Melissa Del Vecchio, Edgar Delbert, Stephanie Goguen, Lucas Mosienko, Brendan Dufault, Brandy Wicklow.

Introduction: Medication adherence can be a challenge for youth (10–18-year) with type 2 diabetes (T2D). We hypothesize that psychological and social factors affect medication adherence.

Methods: A cross-sectional analysis evaluated self-reported adherence to oral and/or injectable diabetes medications in 190 youth with T2D from the improving Renal Complications in Adolescents with Type 2 Diabetes through REsearch cohort (iCARE)using validated questionnaires. Youth were classified as "more adherent" if they reported taking their medications ≥ 5 - 7 days a week or "less adherent" if < 5 days a week. Univariate associations with distress, perceived stress, resiliency, social support, and food security were assessed. Co-variates included age, sex, HbA1c, BMI z-score and diabetes duration.

Results: We found 55(28.9%) of participants were on oral medications only, 90(47.4%) on injectable medications only and 45(23.7%) on both. 57.7%, 52.6% and 52.5% of those on oral medications, injectable medications and both were classified as more adherent respectively. In the less adherent group of the participants on both medications, HbA1c was higher (9.82% vs 9.00%), however, there was no association with reported distress (p=0.21), perceived stress (p=0.995) or resiliency (p=0.35). Social support (p= 0.049) and food security (p=0.019) were positively associated with adherence

Conclusion: Social support and food security are associated with adherence to medication regimens. There was no significant association between mental health measures and medication adherence in our cohort. Additional strategies to support youth with T2D are required.

Abstract 4

Type-2 Diabetes Risk in Offspring: standardization of an exposure model in mice.

Thiago H. L. Baltus, Maryana Kutuzova, Kristin L. Hunt, and Christine A. Doucette

Introduction: In human cohort studies, it has been shown that exposure to type 2 diabetes (T2D) increases the risk for T2D development in exposed offspring 16 times; 4-fold greater than exposure to gestational diabetes mellitus. Currently, we have little mechanistic understanding of how exposure to T2D during gestation drives the susceptibility of offspring to T2D, largely due to limitations associated with human studies and lack of an appropriate rodent model of T2D in pregnancy. Our goal is to characterize a model of T2D in pregnancy and examine the metabolic parameters in the T2D-expsoed offspring.

Methods: To induce T2D pre-pregnancy, C57BL6/J female mice were fed a high-fat and-sucrose (HFS) diet for 8 weeks followed by a single-dose of Streptozotocin (STZ; 75mg/kg) to hinder beta cell compensation causing insulin insufficiency. Control females were fed a standard chow diet and were injected with citrate buffer. All females were bred with healthy male mice and pregnancy established. All offspring were weaned onto a chow diet and evaluated at 4-12 weeks of age for glucose tolerance and insulin secretion capacity.

Results: While on-going, preliminary data indicates that exposure to T2D impacts early life glucose tolerance and insulin secretion in a sex-specific manner.

Conclusions: While in the early stages, our study demonstrates that exposure to T2D during gestation triggers mild metabolic dysfunction in early life, which is more severe in males. Further investigations are needed to understand the mechanistic drivers of these metabolic changes and to determine how this influences susceptibility to subsequent metabolic stress and T2D development.

Abstract 5

Novel knock-in mouse models of prohibitin-1 revealed its role in sex-related differences in kidney biology

Yeshika Bhatia, Niloofar Beheshti Dehkordi, Katherine Bernier, Kinnari Shah, Suresh Mishra

Introduction: Prohibitin-1 (PHB1) is an evolutionarily conserved pleiotropic protein. Recent findings in our laboratory from transgenic mouse models of PHB1 (PHB1-Tg) and a phospho-mutant form of PHB1Y114F (m-PHB1-Tg) have revealed its role in interrelated sex differences in adipose and immune functions in physiology and pathophysiology. This involves two interconnected post-translational modifications of PHB1 (i.e., the palmitoylation at Cys69 site and the phosphorylation at Tyr114 site) previously identified in our laboratory. However, it is not known whether PHB1 has a role in sex-related differences in other cell, tissue, and organ types.

Methods: To gain new insights and to further explore sexually dimorphic pleiotropic attributes of PHB1 at the systemic level, we developed Phb1C69A and Phb1Y14F knock-in mouse models separately using state-of-the art CRISPR/Cas9 technology

Results: The Phb1C69A and Phb1Y114F mice displayed both similarities and dissimilarities in sex-related differences in their immunometabolic phenotypes. Interestingly, sex-related differences in their kidney size were apparent in Phb1 knock-in mice when compared with age- and sex-matched wild-type mice. The male Phb1Y114F mice had significantly larger kidney than the male Phb1C69A mice and wild-type mice, as well as their female counterparts. Further analysis of kidney from the knock-in mice showed structural differences in glomeruli and tubules correlating their size differences. Moreover, analyses of kidney lysates by immunoblotting revealed sex-specific differences in the levels of K48- and K63-polyubiquitinated proteins suggesting sex-related differences in ubiquitin-proteasome system in Phb1 knock-in mice, which may have contributed to sexually dimorphic kidney phenotype.

Conclusion: Initial phenotypic characterization of the Phb1 knock-in mouse models further revealed a role of PHB1 in sex-related differences in kidney biology. As sex differences are known to exist in the structure, physiology, pathophysiology, and in age-related decline in kidney function, the Phb1 knock-in mouse models have created new opportunities and research directions to advance our understanding in this field.

Abstract 6

The endocannabinoid mediator prostaglandin F2α ethanolamide and its pharmaceutical analog Bimatoprost new role: Rolling preadipocyte proliferation

Besma Boubertakh, Olivier Courtemanche, David Marsolais, Vincenzo Di Marzo, Cristoforo Silvestri

Introduction: Prostaglandin F2α ethanolamide (PGF2αEA) is an endogenous molecule. Its pharmaceutical analog glaucoma medication Bimatoprost presents the side effect of decreasing fat amount around the eye. This triggered our curiosity to study PGF2αEA role and Bimatoprost potential as an obesity therapy. White adipose tissue regulation is key to metabolic health, yet still perplexing. The chief endocannabinoid anandamide metabolite, PGF2αEA inhibits adipogenesis, that is, the formation of mature adipocytes. We observed that adipocyte progenitor cells—preadipocytes—following treatment with PGF2αEA yielded larger pellet sizes. Thus, we hypothesized that PGF2αEA might augment preadipocyte proliferation. The objectives are the evaluation of PGF2αEA and Bimatoprost potential to induce preadipocyte proliferation, and the establishment of the underlying mechanism.

Methods: To check the effect of our treatments on preadipocytes, we deployed the 3T3-L1 in cellulo model. We implemented cell viability MTT and crystal violet assays, cell counting, and 5-bromo-2′-deoxyuridine incorporation in cell proliferation ELISA analyses. Additionally, we conducted qPCR and flow cytometry assays to assess our drugs' effects on cell cycle progression.

Results: The conducted assays confirmed our prediction that PGF2αEA and Bimatoprost are inducers of preadipocyte multiplication. They promoted cell cycle progression through suppression of the expression of cell cycle inhibitors p21 and p27. Enticingly, their concentrations that showed no visible effect on cell proliferation or basal transcriptional activity of peroxisome proliferator-activated receptor gamma could, in contrast, reverse the anti-proliferative and peroxisome proliferator-activated receptor gamma-transcription activating effects of Rosiglitazone. MTT and luciferase reporter examinations supported this finding. Importantly, we discovered the implication of the mitogen-activated protein kinase pathway in these effects, as they were blocked by the selective mitogen-activated protein kinase kinase (MAPKK) inhibitor; PD98059.

Conclusion and importance: PGF2αEA is a pivotal regulator of white adipose tissue plasticity, via controlling the preadipocyte pool, and Bimatoprost is a promising candidate for obesity and associated disorders therapy.

Abstract 7

Omega-3 fatty acids modify monocyte energy metabolism through mitochondrial bioenergetic rewiring

Michael J. Byun, Roni Armon, Tamaris Souza, Hope D. Anderson, Ayesha Saleem, Samantha D. Pauls

Background: Chronic inflammation is a driving factor in metabolic diseases like obesity and type 2 diabetes. This heightened immune activation, spearheaded by innate immune cells such as monocytes, has been associated with enhanced glucose metabolism, including oxidative phosphorylation. A recent clinical trial showed that supplementation with the omega-3 fatty acid α-linolenic acid (ALA) reduced oxidative phosphorylation rates in circulating monocytes from women with obesity. However, the mechanism(s) remain unknown.

Objective: Therefore, our objective was to replicate the findings in a cell culture model to explore the molecular mechanism.

Methods: THP-1 monocytes were treated for 48h with 10-40 μM of fatty acid, with a bolus dose at 24h. The Seahorse XFe24 and Oroboros O2k Oxygraph instruments were used to approximate catabolic rates (oxidative phosphorylation and glycolysis) with either glucose or palmitic acid provided as a metabolic substrate. Pro-inflammatory cytokine (IL-1β) level was measured by ELISA. Finally, gene expression was assessed by reverse-transcriptase quantitative polymerase chain reaction.

Results: ALA reduced mitochondrial ATP production by ~24% and increased glycolytic ATP production by ~62% in the presence of glucose. ALA also decreased fatty acid oxidation to a similar extent. Unexpectedly, another omega-3 fatty acid, docosahexaenoic acid (DHA) had similar effects on glucose catabolism. Both ALA and DHA treatment reduced IL-1β levels compared to vehicle, ~63% and 42%, respectively. Finally, we identified pyruvate dehydrogenase kinase 4 (PDK4), an enzyme that inhibits the conversion of pyruvate to acetyl-CoA, as a possible mechanistic candidate. It was significantly upregulated by DHA (~7-fold), though only slightly by ALA (~1.4 fold).

Conclusion: Overall, ALA and DHA similarly dampened oxidative phosphorylation rates and suppressed pro-inflammatory cytokine production. Although only DHA significantly elevated PDK4 levels. This is an important step towards understanding how intervention strategies with omega-3 fatty acids could help treat or prevent chronic metabolic diseases relevant to children and youth.

Abstract 8

Novel Knock-In Mouse Models of Prohibitin-1 Display Amplified Sex-Related Differences in Brown Adipose Tissue

Niloofar Beheshti Dehkordi, Yeshika Bhatia, Suresh Mishra

Background: Adipose tissue-specific transgenic and knockout mouse models of prohibitin-1 (Phb1) have established its role in white and brown adipose tissue (WAT and BAT) biology, including sex-related differences. Sex differences in BAT biology are known to exist in both humans and rodents, suggesting that PHB1 may play a significant role in mediating these differences.

Methods: To gain new insights and further explore the role of PHB1, we developed Phb1C69A and Phb1Y114F knock-in mouse models using state-of-the-art CRISPR/Cas9 technology. These models specifically lack two key post-translational modification (PTM) sites in PHB1. The knock-in mice were studied to observe sex-related differences in BAT through gross anatomical examination, histological analysis, and transmission electron microscopy (TEM).

Results: The Phb1 knock-in mice displayed clear sex-related differences in their BAT. Gross anatomical analysis showed that BAT from both female knock-in mice exhibited a more pronounced brown coloration compared to their male counterparts and to male and female wild-type mice. Histological and TEM analyses revealed that brown adipocytes in the BAT of female knock-in mice were smaller in size but greater in number compared to male knock-in mice. Additionally, there were amplified sex-related differences in the distribution of multilocular lipid droplets between male and female knock-in mice, with improvements seen in the BAT structure of female knock-in mice. Among the three genotypes, the female Phb1Y114F mice demonstrated enhanced BAT structure, whereas the male Phb1C69A mice showed an opposite effect.

Conclusion: The novel Phb1 knock-in mouse models, which display amplified sex-related differences in BAT, have opened new avenues for understanding the complex roles of PHB1 in adipose tissue biology. These findings highlight the importance of PHB1's post-translational modifications in regulating sex-specific characteristics of BAT, providing a valuable tool for further research into sex differences in adipose tissue function.

Abstract 9

Feasibility of a Virtual Dialectical Behaviour Therapy Program for Youth Living with Type 2 Diabetes in Manitoba, Canada.

Melissa Del Vecchio, Lily Pankratz, Leslie E. Roos, Emily E. Cameron, Elizabeth Sellers, Jon McGavock, Mandy Archibald, Linda Diffey, Laurence Y. Katz, Tanya Dawn McDougall, Lionel Mason, Allison Dart, Brandy Wicklow

Background: Youth living with type 2 diabetes (T2D) have identified mental health as an important factor in diabetes management. Dialectical Behavior Therapy (DBT) is an evidence-based program that includes teaching mindfulness, emotion regulation, and distress tolerance. This pilot project examines the feasibility and tolerability of virtual DBT skills training as a mental health intervention in youth with T2D.

Methods: Youth (14-22 years old) were approached by email, telephone, or in-person. Individuals were screened for eligibility using the Kessler-6 (K6) and Patient Health Questionnaire-9 (PHQ-9). Eligibility included a diagnosis of T2D and clinically elevated mental health symptoms (K6 of 5-13 and PHQ-9 of 5-19) . Recruitment, enrollment, and adherence rates to the DBT program were determined.

Results: Of the youth approached, 34/76 (44.7%) completed screening. Screened youth were 50% female with a mean age of 15.9 (SD=1.83) years. Mean K6 and PHQ-9 scores were 10.2 (5.32) and 13.2 (7.76) respectively. 73.5% (25/34) of screened youth required a follow-up with the psychology team. Thirteen of 18 eligible youth were enrolled. Eight youth (61.5%) completed the 16-week program. Feasibility was impacted by lack of access to Wi-Fi and functional devices and challenges in finding group times that worked for youth.

Conclusion: Successful enrollment and retention rates were low. Logistical barriers to enrollment and completion of the DBT sessions need to be further assessed to improve uptake to the full DBT training.

Abstract 10

The effectiveness of new Urban Trail Infrastructure on Physical Activity and Active Transportation: A Systematic Review and Meta-Analysis of Natural Experiments

Isaak Fast, Nashed, Lötscher, Klaprat, Askin, De Visser, Jon McGavock

Background: Cities are investing billions of dollars in new cycling infrastructure to support active transportation (AT) and physical activity (PA). Little empirical evidence exists describing the effectiveness of this infrastructure.

Methods: We searched CINAHL, EMBASE (Ovid), MEDLINE (Ovid), SPORTDiscus, TRD/Transportation Research Information Services (TRIS), Web of Science and Google Scholar for articles published from 2010 to 2023. We included studies with experimental pre-post designs that reported PA, or trail counts for an intervention and control area. The interventions were limited to protected urban trails. Primary outcomes were individual physical activity (PA) and trail use counts. A modified risk of bias tool will be employed to assess the methodological quality of selected studies (Prospero ID: CRD42023438891).

Results: Three independent reviewers screened abstracts from 3936 articles. 25 articles describing natural experiments of the effect of adding urban trails on changes in PA or AT were included: 12 studies (n=11,464) measured changes in PA, 8 studies measured changes in cycling traffic and 5 studies (n=4,957,696) measured changes in AT/bike use. Meta-analysis revealed that new trails increased PA among individuals living close to a trail, compared to those living far from a trail (SMD = 0.12; 95% CI: 0.04, 0.20; I2 = 73%). This effect was marginally stronger when restricted to individuals living closest to trails (SMD = 0.14; 96% CI: 0.06 to 0.25, I2 = 74%; n = 8234). No natural experiments to date have researched these impacts on children. All studies were at high risk of bias due to a failure to adhere to reporting guidelines for quasi-experimental studies.

Conclusions: The addition of protected cycling infrastructure appears to increase PA and rates of AT for individuals living in neighbourhoods that receive them. The strength of this evidence could be enhanced with the application of principles of causal inference and increased racial, gender and socio-economic diversity of populations that receive new cycling infrastructure.

Abstract 11

Acute secreted protein acidic and rich in cysteine (SPARC) injections to mice is towards metabolic benefits and potential long-term antidiabetic effects

Abdelaziz Ghanemi, Mayumi Yoshioka, Jonny St-Amand

Background: We have previously shown that secreted protein acidic and rich in cysteine (SPARC) overexpression (transgenic mice) and exercise-induced SPARC (in mice) improves the metabolic profile as well as the muscle performance including glycemia-related patterns.
As those publications represent studies we have performed over months, they reflect chronic effects of SPARC. In this study we investigated the acute effect of SPARC injection in mice.

Methods: First, we validated the injection model and confirmed that intraperitoneal SPARC injections to C57BL/6J mice increased SPARC serum levels. Second, we identified 4 hours as the optimum time with the highest SPARC serum level increase following the injection (western blot analyses).
Finally, to study SPARC injection effects, 6 male and 6 female C57BL/6J mice were purchase at 7 weeks old. At the age of 12 weeks, we divided them into 4 groups (male injected with SPARC, male injected with saline, female injected with SPARC and female injected with saline). 4 hours after the injections the mice were sacrificed and various biological studies/measures were conducted: Metabolic and functional proteins expression, glycemia and organs/tissues weights.

Results: Injection of SPARC in mice activated pathways of extracellular matrix remodeling, glucose metabolism and mitochondrial biogenesis in the skeletal muscle. It also supressed the pathway of muscle protein degradation which may in turn activate myogenic differentiation. There was no effect on pathways of protein synthesis. In addition, sexual differences and/or interaction between SPARC and sex were found in several pathways such as extracellular matrix remodeling, mitochondrial biogenesis, muscle protein degradation and myogenesis. For the glycemia and organs/tissues weights, we had sex effect but no SPARC effect.

Conclusions: Our results point direct and indirect metabolic benefits SPARC injection could have at the metabolic and functional levels that can help identify new pathways, therapies and therapeutic targets for diabetes among other metabolic disorders.

Abstract 12

The Impact of Omega-3 fatty acids on glucose metabolism in macrophage cell models

Floriane Houenagnon, Michael Byun, Tamaris Souza, Ayesha Saleem and Samantha Pauls

Introduction: Obesity is associated with a state of chronic inflammation, which is a risk factor for progression to diseases such as Type 2 Diabetes. Macrophages play a key role in obesity-associated inflammation. Common n-3 PUFA, such as docosahexaenoic acid (DHA) and α-linolenic acid (ALA), have anti-inflammatory effects. In monocytes, this is associated with changes to catabolic pathways. Their impact on metabolic pathways in macrophages remains unclear.

Objective: To describe the effects of n-3 PUFA on glucose metabolism by glycolysis and mitochondrial respiration in macrophages.

Methods: Macrophages, including murine RAW 264.7 cells and human THP1-derived macrophages (TDM), were treated with the following: Vehicle, DHA, ALA, and Oleic acid (OA, a monounsaturated fatty acid control) for 24 hours. Then, the ATP rate assays were conducted using a Seahorse XFe24 instrument. Data were normalized by measuring protein content via BCA assay or by cell counting using the Cytation 5 cell imaging multimode Reader. Cell viability was assessed using the CYQUANT XTT assay.

Results: TDM and RAW macrophages have different metabolic phenotypes. Examining vehicle-treated conditions shows that the ratio of total ATP produced from mitochondrial respiration to glycolysis is ~15:1. In RAW 264.7 cells, that ratio is ~ 1:1. In TDM, DHA significantly increases the percent of ATP derived from glycolysis. In RAW 264.7 cells, neither n-3 PUFA significantly altered the balance of catabolic pathways. The XTT assay showed that DHA significantly increased TDM viability by approximately 25%, highlighting the importance of accurate normalization of Seahorse data.

Conclusion: The results highlight the heterogeneity between the cell lines. Further comparison of the basal metabolic differences between macrophage models will allow us to see which cell line model best mimics the primary macrophage cells.

Abstract 13

Docosahexaenoic Acid Differentially Modulates the HMG CoA Reductase Pathway of Endothelial Cells Dependent Upon Growth State: Implications for Cardiovascular Health and Diabetes Care

Shiqi Huang, Peter Zahradka, Carla Taylor

Introduction: Atherosclerosis, usually initiated by endothelial dysfunction, leads to major life-threatening diabetes complications. Docosahexaenoic acid (DHA) has been traditionally viewed as athero-protective, but recently clinical trials on omega-3 fatty acids and cardiovascular disease (CVD) have produced mixed results. We previously found that DHA differentially regulated endothelial nitric oxide synthase in growing versus quiescent endothelial cells, which approximate the dysfunctional and healthy states in vivo, respectively. Thus, we hypothesized that DHA could benefit healthy endothelial cells but not dysfunctional ones.

Methods: RNA-seq was done on DHA-treated (20 or 125 μM for 8 h) and control human EA.hy926 cells in both states. The data were processed by the RSEM-STAR-DESeq2 pipeline. DiUerentially expressed genes (DEGs) were subjected to pathway analysis with clusterProfiler. Genes selected from candidate enriched pathways were validated by Western blotting.

Results: Principal component analysis showed distinct groupings based on cell growth state and DHA concentration. DESeq2 identified 104 and 173 DEGs unique to growing and quiescent cells, respectively, at 20 μM DHA. Pathway analysis revealed significant enrichment for cholesterol biosynthesis-related terms of downregulated DEGs, including HMGCR, SREBF2, and INSIG1, in quiescent cells treated with 20 μM DHA, while SREBF1 was downregulated in both states. Moreover, many genes associated with the Rho GTPase pathway, downstream of HMG CoA reductase (HMGCR), were downregulated by 20 μM DHA only in quiescent cells.

Conclusions: These results attest to similarities between the eUects of DHA and statins, inhibitors of HMGCR that address CVD via both cholesterol reduction and pleiotropic actions on Rho GTPase. Our study reveals that only quiescent endothelial cells respond positively to DHA. It pioneers a novel perspective that DHA should be targeted for CVD prevention before patients develop severe endothelial dysfunction. Further studies are required to validate the in vivo responses to DHA, especially its role in preventing cardiovascular complications in diabetes.

Abstract 14

The effect of the HNF1-α G319S polymorphism on kidney health of children in the Next Generation cohort exposed to type 2 diabetes in utero

Priscilla Irabor, Allison Dart, Elizabeth Sellers, Stephanie Goguen, Yash Rawal, Brandy Wicklow

Introduction: Exposure to type 2 diabetes (T2D) in utero is associated with long-term risk of chronic kidney disease. The HNF1-α G319S polymorphism increases the risk of developing T2D, however, associations with kidney outcomes prior to diabetes onset is unknown. We sought to evaluate kidney health in children exposed to T2D in utero with and without the HNF1-α G319S polymorphism.

Methods: This is a cross-sectional analysis of participants from the Next Generation cohort who were exposed to T2D in utero, normoglycemic and 5 to 11 years old. Outcomes (glycosuria, random urine albumin: creatinine ratio (ACR), albuminuria status (ACR>3mg/mmol) and hypertension status) were analyzed using descriptive statistics.

Results: A total of 142 participants were included in the analysis (52.1% female, 8.98±1.91 years, median BMIz {2.20[1.73, 2.46]}, median Hemoglobin A1C {5.50[5.30, 5.70]}). The wildtype group (n=67) had 4 participants with albuminuria while the variant group (n=75) had no one with albuminuria. None of the participants in either group tested positive for glycosuria. Median ACR was (0.50 [0.30, 0.92] vs 0.60 [0.30, 0.90], p = 0.616) and hypertension rates were 57.4% vs 61.0%, (p = 0.843) in the wildtype and variant group respectively.

Conclusion: We observed no glycosuria and low rates of albuminuria in the cohort. There were high rates of hypertension in both groups, which requires further investigation to determine its association with exposure to T2D in utero.

Abstract 15

A patient-centred approach to designing a randomized controlled trial of a novel peer-led intervention for adolescents with type 1 diabetes (T1D). A descriptive case study.

Andrea MacIntosh, TEAM Trial Patient Partners, Jon McGavock

Introduction: The interest in designing and piloting lay-person led interventions is growing with medical communities acceptance of the importance of lived experience. The aim of this study is to describe the patient-led approach to designing a behavioural intervention to support adolescents living with T1D and describe the process of training young adult peers to deliver the intervention.

Methods: We followed CIHR's framework for patient engagement, integrating patient co-researchers throughout all aspects of the project with a focus on CIHR's core principles for engagement: Inclusiveness; Support, Mutual Respect and Co-Building the trial. Patient partners were involved as co-PI's on the funded grant, the studies conducted that informed the trial and were included in the governance and decision making for the trial. A virtual Hackathon and planning meetings were conducted to co-design elements of the intervention and the peer mentor training.

Results: After securing a four-year operating grant (CIHR-JDRF) to pilot the trial, we spent 4 months planning and designing a training program for young adult peer mentors. Mentors aged 21-30 years living with T1D who reported meeting physical activity guidelines were recruited to work as mentors in Winnipeg and Mississauga. Patient partners were involved in interviewing, hiring and training 3 mentors per site. Over a period of 8 months, two in-person gatherings with patient partners and mentors fostered skills in motivational interviewing (MI), validation talk, safe PA and strategies for behavioural change. Mentors completed additional virtual training in MI, and mental health first aid and are now actively recruiting families into the trial. The model of patient-led training was well received by all members of the team.

Conclusions: Using CIHR's model for respectful patient engagement, we successfully designed, planned and delivered training for young adults living with T1D to confidently deliver a novel behavioural intervention for youth living with T1D.

Abstract 16

Amyloid-Associated Shift in the Phenotype of Resident Macrophages in Human Islets – A Potential Mechanism for Islet Inflammation in Type 2 Diabetes

Danish Malhotra, Janessa Sawatzky and Lucy Marzban

Background: The incidence and prevalence of type 2 diabetes (T2D) are increasing in Manitoba, Canada, and worldwide, not only in adults but also in children. In patients with T2D, formation of toxic protein aggregates named amyloid in pancreatic islets contributes to islet inflammation, and beta-cell dysfunction and death, by promoting IL-1beta production and activation of the Fas-mediated apoptotic pathway. Islet macrophages are the main source of amyloid-induced IL-1beta production in human islets. In this study, we examined the potential effects of amyloid formation on pro-inflammatory (M1) and anti-inflammatory (M2) islet resident macrophages.

Methods: Human islets (n=4 cadaveric donors) were cultured in normal glucose as control (5.5 mM; no or minimal amyloid formation) or elevated glucose (11.1 mM; islets form amyloid) for 7 days. Quantitative immunolabelling was performed on paraffin-embedded human islet sections for insulin and each CD68 (general macrophage marker), iNOS (M1 marker), CD163 (M2 marker), IL-1beta, Fas, thioflavins S (amyloid), or TUNEL (apoptosis). Also, IL-1beta release from human islets was assessed.

Results: Human islets progressively formed amyloid during 7-day culture in elevated glucose (but not in normal glucose), leading to increased number of apoptotic beta-cells. Amyloid formation was associated with elevated islet IL-1beta levels, upregulation of the Fas cell death receptor in beta cells, and a shift in the phenotype of islet resident macrophages, manifested as elevated iNOS-positive (M1) and reduced CD163-positive (M2) macrophages. The mean of total macrophages per islet was comparable in pre-culture and 7-day cultured islets at both glucose levels.

Conclusion: Amyloid formation in human islets led to a shift in macrophage phenotype towards pro-inflammatory (M1), thereby promoting IL-1beta production and IL-1bteta/Fas-mediated beta-cell apoptosis. Thus, modulation of islet macrophage phenotype may provide a therapeutic strategy to improve beta-cell survival in children and adults with T2D by reducing amyloid-induced beta-cell death.

Abstract 17

Kidney Biopsies in Diabetes: A Comparative Study of Youth and Adults

Jasmine Manji, Bryce Barr, Elizabeth Sellers, Oksana Harasemiw, Ian Gibson, Navdeep Tangri, Brandy Wicklow, Allison Dart

Background/Introduction: **Y**outh with type 2 diabetes (T2D) are at high risk of kidney failure. We sought to evaluate differences in biopsy findings between youth and adults with diabetes in Manitoba that might explain differences in rates of progression. We hypothesized that youth with diabetes have more non-diabetic kidney pathology than adults.

Methods: This retrospective study utilized a glomerular disease biopsy report registry linked to the Manitoba Centre for Health Policy (MCHP) from 2002-2021. Biopsies from adults (age 19-40) with type 1 and type 2 diabetes were included. Additional biopsy reports from adults 2022-23 and youth ≤ 18 years from 2002-23 were manually evaluated. Clinical data was extracted from MCHP (adults) and clinical charts (youth). Pathological features evaluated included primary diagnoses, diabetes-related changes, chronic structural damage, and immunofluorescence. Clinical covariates included sex, age, diabetes duration, hemoglobin A1c, (HbA1c), estimated glomerular filtration rate (eGFR), urine albumin:creatinine ratio (ACR). Descriptive statistics were performed.

Results: A total of 153 adult and 34 youth biopsies were included. Mean age at biopsy and mean age of diabetes diagnosis was 32±6 and 26±8 years for adults and 15±2 and 11±3 years for youth. Median diabetes duration was shorter in youth (2.8 (1.3-4.9) vs 5.0 (1.0-10.0) years, p=0.0004) and adults had better glycemic control (A1c 7.6±2.3 vs 10.3±2.8, p<0.0001). Adults had more albuminuria (median ACR 330.0 (172.3-591.5) vs 94.0 (34.9-204.8) mg/mmol, p<0.0001) and lower eGFR (median 37 (14-79) vs 143 (127-167) ml/min/1.73m2, p<0.0001). Adults had more diabetic nephropathy (43.8% vs 26.5%, p=0.01) whereas youth had more non-diabetic diseases, including non-proliferative glomerulonephritis (29.4% vs 13.7%, p=0.05).

Conclusions/Importance: There are differences in clinical status and biopsy findings between adults and youth with diabetes undergoing clinical kidney biopsies. Youth are more likely to have non-diabetic kidney diseases whereas adults have more diabetic nephropathy and chronic renal parenchymal scarring. Further studies needed to examine eGFR trajectories based on biopsy findings to better understand clinicopathologic implications.

Abstract 18

Gestational Diabetes Mellitus Induces Cardiac Dysfunction and Altered Mitochondrial Protein Acetylation in the Offspring Heart

Caitlin Menzies, Mateusz M Tomczyk, Bo Xiang, Stephanie M. Kereliuk, Richard Leduc, Vernon W. Dolinsky

Background/Objective: Exposure to intrauterine gestational diabetes mellitus (GDM) increases risk for cardiovascular disease in offspring later in life. Previously we have found that mitochondrial dysfunction contributes to the development of cardiomyopathy in GDM offspring, partially attributable to abhorrent protein lysine acetylation. Protein lysine acetylation is a major regulatory mechanism of cardiac mitochondria that regulates activity of metabolic enzymes. Sirtuin-3 (SIRT3) is the main mitochondrial deacetylase protein known to be downregulated in GDM and by chronic high fat diet. Our work elucidates the influence of intrauterine GDM exposure and postnatal diet on cardiac development in offspring and evaluate the role of SIRT3 within this context.

Methodology: GDM was induced by feeding female mice a high fat and sucrose (HFS; 45% fat) diet for 6 weeks prior to mating and throughout pregnancy and lactation. Control lean dams were fed a low fat (LF; 10% fat) diet. Dams were mated to transgenic male sires overexpressing cardiac SIRT3 (SIRT3-TG) to generate a mix of non-transgenic and transgenic offspring. Postweaning, offspring from Lean and GDM dams were fed LF and HFS diets. Echocardiography was performed in 15-week-old offspring. Acetylated mitochondrial peptides were extracted from offspring hearts via immunoprecipitation and quantified by mass spectrometry

Results: Cardiac hypertrophy and diastolic dysfunction were found in tandem with alterations to the cardiac mitochondrial acetylome among non-transgenic offspring exposed to intrauterine GDM. SIRT3-TG offspring did not display hypertrophic phenotypes observed in their non-transgenic littermates, suggesting a protective role of cardiac SIRT3 overexpression against GDM- and HFS-induced cardiac hypertrophy in both male and female offspring.

Conclusion: Cardiac mitochondrial enzyme acetylation represents a novel molecular mechanism that contributes to GDM-induced mitochondrial dysfunction and cardiovascular disease in offspring. Our findings support the idea of SIRT3 as a potential therapeutic target against the development of cardiovascular disease in GDM-exposed offspring.

Abstract 19

Estradiol promotes neurite outgrowth in adult sensory neurons through AMPK/ATF3 signaling pathway

Pranav Mishra, Benedict C. Albensi, Paul Fernyhough

Introduction: Estrogen can affect neuropathic pain by enhancing axonal outgrowth of dorsal root ganglion (DRG) sensory neurons. Adult rat DRG sensory neurons express both estrogen receptors (ERs) a and b. 17-beta estradiol (E2), the most potent form of estrogen, regulates development, survival, and axonal outgrowth of these neurons. Cellular energy sensor AMPK can regulate the expression of both activating transcription factor-3 (ATF3), involved in neuronal regeneration and peroxisome proliferator-activated receptor g coactivator-1a (PGC-1a), involved in mitochondrial biogenesis. The objective of the present study was to investigate the impact of E2 treatment and gain a comprehensive understanding of the ER signaling pathway in DRG sensory neurons.

Methods: DRG neurons derived from adult SD rats were cultured under defined conditions. Subsequently, the cultured neurons were treated with E2. Expression levels of pAMPK, ATF3, and PGC-1α were determined using western blotting and mitochondrial function was assessed using Seahorse XF24. DRG neurons were stained with β-tubulin III and images were analyzed using ImageJ to evaluate total neurite outgrowth. We also blocked AMPK and CAMKKb using Compound C and STO-609 respectively; to investigate their effects on ATF3 and axonal sprouting.

Results: E2 treatment increased the levels of phosphorylated-AMPK, ATF3, PGC-1a and ETC Complex-I. E2 also elevated total neurite outgrowth and basal respiration. Inhibiting AMPK using compound C also inhibited E2-mediated increases in ATF3 expression and neurite outgrowth, suggestive of AMPK acting upstream of ATF3. Blockade of CAMKKβ (upstream activator of AMPK) using STO-609, caused inhibition of E2-mediated AMPK activation confirming that E2 activated AMPK via the CAMKK pathway. Our work with ERa and ERb agonists revealed that these effects were mediated via ERa.

Conclusion: This study unveils that E2 acts through ERa to promote neurite outgrowth via a pathway involving activation of CAMKKb/AMPK and ATF3 in adult DRG neurons and highlights its potential therapeutic applications in alleviating neurodegenerative diseases, including peripheral neuropathy.

Abstract 20

Maternal Resveratrol (RESV) Supplementation and the Effects on Cardiac Hypertrophy, Mitochondrial Metabolism, and Calcium Transport

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

Background/Introduction: Gestational diabetes mellitus (GDM) is a condition that manifests in pregnancy and is characterized by insulin resistance, glucose intolerance, and hyperglycemia, and impact maternal and offspring health. Medications have shown effectiveness but have associated risk of adverse pregnancy outcomes and the long-term effects on the offspring are unknown. Our previous studies in rats observed that cardiomyocytes of GDM-offspring exhibit hypertrophy, mitochondrial dysfunction, and impaired calcium flux. In this study, we hypothesize that administration of Resveratrol (RESV) to maternal GDM diet will mitigate mitochondrial dysfunction, cardiac hypertrophy and improve calcium flux in GDM-exposed offspring.

Methods: Female Sprague-Dawley rats were fed a low-fat (Lean, 10% kcal fat) or high-fat and sucrose (GDM, 45% kcal fat) diet six weeks before mating to induce GDM. A subgroup of GDM dams were switched to a diet containing RESV (GDM+RESV, 45% kcal + 4g/kg RESV). At e18.5 fetal echocardiography was performed to assess cardiac structure. To determine the effects of RESV on GDM-offspring, e20 pups were sacrificed for fetal cardiomyocyte isolation. Measurements of mitochondrial respiration were performed using the Agilent-Seahorse XFe24. Measurements of calcium flux were performed using fluo-4 on the Cytation-5.

Results: Fetal echocardiography revealed maternal RESV attenuated GDM-induced cardiac hypertrophy. GDM-exposed offspring showed larger intraventricular septal and left ventricular posterior wall thickness compared to lean and GDM+RESV offspring. Cardiomyocytes isolated form GDM-offspring had decreased mitochondrial respiration, but higher glycolytic activity compared to lean and GDM+RESV offspring suggesting a metabolic switch in offspring cardiomyocytes. Furthermore, cardiomyocytes isolated from GDM-offspring exhibited delayed calcium flux cycles compared to lean and GDM+RESV offspring upon angiotensin II stimulation.

Conclusion/Importance: Our data replicates the previous findings that GDM-offspring exhibit cardiac hypertrophy and mitochondrial dysfunction. Maternal RESV supplementation improved mitochondrial respiration which contributed to impaired calcium flux upon angiotensin II stimulation. Importantly, maternal RESV supplementation attenuated GDM-induced cardiac hypertrophy in GDM-offspring.

Abstract 21

SIRT3 Deficiency in the Liver Results in Hepatic Steatosis and Adipocyte Expansion in Gestational Diabetes

Jewel Paskaruk, Khushali Trivedi, Bo Xiang & Vernon Dolinsky

Introduction: Gestational diabetes mellitus (GDM) is the most common pregnancy complication, affecting around 15-20% of pregnancies at the time of delivery in Canada, with obesity being a major risk factor. Fat accumulation in the liver contributes to insulin resistance, which is characteristic of GDM. White adipose tissue expands during pregnancy; however, adipocyte hypertrophy can have negative implications for insulin sensitivity. This project investigates the role of Sirtuin 3 (SIRT3), a mitochondrial protein deacetylase that is important in energy pathways such as fatty acid oxidation during pregnancy. **We hypothesize that SIRT3 deficiency in the liver induces hepatic steatosis and adipocyte expansion during pregnancy.**

Methods: Mice with liver-specific-deletion of SIRT3 (SIRT3-LKO) were generated by crossing Sirt3tm1.1Auw mice from Jackson Labs with loxP sites flanking exons 2-3 of the Sirt3 gene with Cre-recombinase mice with an albumin-promoter. SIRT3-LKO mice and Cre-negative controls fed either low fat diet (10% kcal fat) or high fat sucrose diet (45% kcal fat) for 6-weeks before pregnancy and throughout the 3-week mouse pregnancy to induce GDM. Pregnant mice were sacrificed at embryonic day 18.5 and liver and gonadal white adipose tissue (GWAT) depots were collected for histological visualization of lipids using hematoxylin and eosin and Oil Red O.

Results: SIRT3-LKO mice exhibited hepatic steatosis compared to controls during pregnancy ($p<0.0001$), assessed by quantifying Oil Red O positive area of liver sections. The diameter and number of adipocytes per GWAT section showed adipocyte expansion in SIRT3-LKO mice compared to controls during pregnancy ($p<0.0001$) (Two-way ANOVA).

Conclusion: Our results show that deficiency of SIRT3 in the liver leads to hepatic steatosis and adipocyte expansion, independent of diet during pregnancy. This may contribute to insulin resistance observed in GDM. Better understanding the role of SIRT3 in the development of GDM could lead to new therapeutics.

Abstract 22

Proinsulin processing is sustained during DNA damage-mediated senesce in adult human β-cells

Camille Prefontaine, Nayara R. Morelli and Peter J. Thompson

Introduction: Recent experiments demonstrate that residual pancreatic β-cells in human donors with type 1 diabetes have impaired proinsulin processing. Beta cells undergo stress responses during the progression and onset of type 1 diabetes, including senescence. How senescence impacts proinsulin processing in human β-cells is not yet understood. We hypothesized that senescence triggered by DNA damage would impair proinsulin processing.

Methods: Islets from human adult donors without diabetes and the fetal-derived EndoC-βH5 human β-cell model were induced into a senescence stress response using the DNA-damaging agent bleomycin. Western blotting was used to quantify relative levels of insulin processing enzymes PC1/3, PC2, CPE, and the endogenous PC1/3 inhibitor ProSAAS in control and senescent islets and EndoC-βH5 cells. Levels of proinsulin and mature insulin content in islets and EndoC cells were measured by ELISAs.

Results: The senescence stress response in donor islets led to increased levels of PC1/3 ($P<0.001$), CPE ($P<0.05$) and ProSAAS ($P<0.05$), whereas PC2 was unchanged ($P=0.115$). In contrast, senescence in the EndoC-βH5 β-cells led to decreased levels of PC1/3 ($P<0.01$), CPE ($P<0.05$) and ProSAAS ($P<0.001$), without a significant effect on PC2 ($P=0.19$). Consistent with the changes in insulin synthesis enzymes, senescent adult islets showed unchanged levels of total proinsulin ($P=0.75$) or mature insulin ($P=0.14$), whereas senescent EndoC-βH5 cells had reduced levels of both proinsulin ($P<0.05$) and mature insulin ($P<0.005$).

Conclusion: These results show that proinsulin processing is sustained during senescence triggered by DNA damage in adult islets. We propose that the senescence stress response may have different impacts on insulin synthesis based on the β-cell maturation stage. This finding has implications for understanding how proinsulin processing may be affected when DNA damage occurs in β-cells of younger versus older individuals who develop type 1 diabetes.

Abstract 23

Effect of Cold Exposure on Lipid Synthesis in Brown and White Adipose Tissue in Mice

Kailey Qin, Christophe Noll, Etienne Mouisel, Khalil Bouyakdan, Eric Rhéaume, Thierry Alquier, Jean-Claude Tardif, Dominique Langin and André C. Carpentier

Background: Brown adipose tissue (BAT) was identified in past studies as a potential therapeutic target to treat obesity due to its ability to increase energy expenditure when exposed to low temperatures. The question that remains is whether cold exposure has a similar effect on white adipose tissue (WAT), which is primarily involved in the pathogenesis of obesity.

Methods: To assess the effect of cold exposure on BAT and WAT lipid synthesis, we measured fatty tissue uptake and retention of 11C-acetate with dynamic micro-positron emission tomography (μPET) in two cohorts of rodents: the first group was on a control diet, and the second was on a high fat, high-cholesterol (HFHC) diet. All rodents were exposed to a 7-day thermoneutral temperature (30oC) and a 7-day cold temperature (10oC) using a random crossover design, separated by a 7-day wash-out period.

Results: As expected, BAT 11C-acetate tissue retention was increased after cold vs. thermoneutral exposure in both groups of mice (n=12, +78% in the control diet group, $P<0.0001$; n=30, +75% in the HFHC diet group, $P<0.0001$). Cold exposure also increased sub-cutaneous inguinal WAT 11C-acetate tissue retention, but to a greater extent in the mice fed the control diet (+87%, $P<0.0001$) than in those fed the HFHC diet (+7%, $P<0.008$). Moreover, we observed a significant increase in epididymal WAT 11C-acetate tissue perfusion in the mice fed the control diet (+32%, $P<0.0001$), but not in those fed the HFHC diet (-10%, $P=0.11$).

Conclusion: Our data suggest that cold-induced increase in BAT lipid synthesis is enhanced, while cold-induced increase in WAT lipid synthesis is blunted in mice fed a HFHC diet.

Abstract 24

Maintenance of prosurvival signaling is independent of secreted GDF15 in senescent human beta cells

Nayara Rampazzo Morelli, Camille Préfontaine, Jasmine Pipella and Peter J. Thompson

Background/Introduction: Beta cell senescence is a stress program involved in Type 1 Diabetes (T1D) in humans. Senescence leads to apoptosis resistance but the mechanisms governing this phenotype in human beta cells are not known. Growth Differentiation Factor 15 (GDF15) is secreted from senescent human beta cells and is known to contribute to beta cell survival in other settings. We hypothesized that secreted GDF15 maintains prosurvival signalling in senescent human beta cells.

Methods: Endogenous senescent beta cells and secreted GDF15 were studied in isolated islets from an adult T1D donor using flow cytometry and ELISA. Induced senescent beta cells and secreted GDF15 were studied in islets from non-diabetic adult donors and EndoC-βH5 human beta cells using a DNA damage protocol. GDF15 was neutralized using a monoclonal antibody. Viability, gene expression and secretomes were measured using a combination of molecular assays.

Results: Senescent beta cells were identified in islets from an adult T1D donor and coincided with high GDF15 secretion as compared to islets from a non-diabetic donor ($P < 0.0001$). Neutralization of secreted GDF15 did not impact induced senescent human islet ($P = 0.2360$) or EndoC-βH5 viability ($P = 0.9744$). However, neutralizing GDF15 led to reduced GDF15 ($P = 0.0107$) and BCL2L1 ($P = 0.0051$) mRNA levels in senescent human islets.

Conclusion/Importance: Together these data suggest that secreted GDF15 is not required for the prosurvival phenotype in senescent human beta cells. Rather, secreted GDF15 exhibits autocrine activity during senescence in human islets to regulate transcriptional responses.

Abstract 25

The association between diabetes in pregnancy and infant feeding practices

Yash R. Rawal*, Priscilla Irabor, Elizabeth Ac Sellers, Brandy A. Wicklow

Introduction: Despite the known benefits of breastfeeding, women experiencing diabetes in pregnancy may face unique barriers to breastfeeding. The purpose of this study was to examine the association between diabetes in pregnancy with infant breastfeeding practices in the Next Generation cohort.

Methods: Cross-sectional analysis of data from maternal-infant dyads in the cohort. Data was collected through hospital records and questionnaires including: maternal diabetes status, gestational age, NICU stay, infant hypoglycemia, birth mode and infant feeding (any vs no breastfeeding). Descriptive statistics and chi square tests and one-way anova were used.

Results: 421 infants were included (50.4% female) with 50.4% exposed to T2D, 24.9% to GDM, and 24.7% to no diabetes. Infants exposed to T2D (39.6%) and GDM (43.8%) were less likely to breastfeed compared to not exposed to diabetes (79.8%) ($p<0.001$). Infants born to mothers with T2D compared to GDM and control were more likely to be premature (T2D-36.8 weeks ,GDM-37.8 weeks, no DM-38.5 weeks, $p<0.001$),delivered by C-section (40% T2D vs 20.8% GDM vs 20.7% no DM, $p=0.001$ and $p=0.002$, respectively), hypoglycemic episodes (44.3% T2D vs 12% GDM vs 3.8% no DM,$p<0.001$) and admitted to the NICU (33.1% T2D vs 6.6% GDM vs 13.8% no DM,$p<0.001$,$p=0.002$, respectively). There were no significant differences in birthweight between groups.

Conclusion: Infants exposed to any diabetes in utero are less likely to breastfeed. This may be in part related to the increased likely hood of c-section delivery, prematurity, and NICU stay. A better understanding of the challenges in breastfeeding is necessary to inform timely, targeted and appropriate interventions.

Abstract 26

Muscle cell SIRT3 activation improves glucose utilization by offspring exposed to gestational diabetes.

Anna Shipylova, Caitlin Menzies, Vernon W. Dolinsky

Background/Introduction: Intrauterine exposure to Gestational Diabetes Mellitus (GDM) is associated with an elevated risk of offspring obesity and type 2 diabetes (T2D) in later life. Skeletal muscle has an important role in regulating blood glucose levels since it is a major site of glucose utilization. Muscle mitochondrial dysfunction is a mechanism that precedes the development of T2D in adults that is partially attributable to reduced expression of SIRT3, the main mitochondrial deacetylase protein. Protein acetylation is a major regulatory mechanism of mitochondria that regulates activity of metabolic enzymes. We hypothesize that increasing SIRT3 in skeletal muscle tissue of offspring may provide protection against GDM-induced metabolic dysfunction.

Methods: To induce GDM female mice were fed a high fat and sucrose (HFS; 45% fat) diet for 6 weeks prior to mating and throughout pregnancy and lactation. Control lean dams were fed a low fat (LF; 10% fat) diet. Dams were mated to transgenic male sires overexpressing SIRT3 (SIRT3-TG) in skeletal muscle tissue to generate a mix of non-transgenic and transgenic offspring. Postweaning, offspring from Lean and GDM dams were fed LF or HFS diets. To evaluate metabolic differences, insulin and glucose tolerance tests were performed.

Results: Non-transgenic offspring exposed to GDM and fed a postnatal HFS diet displayed significantly higher body weight, fasted blood glucose, impaired response to insulin, and impaired glucose tolerance; these differences were not observed in GDM-HF-SIRT3 TG offspring, suggesting a protective effect of SIRT3. Western blot analysis of gastrocnemius muscle revealed increased SIRT3 in our SIRT3-TG offspring compared to WT littermates. We found no differences in citrate synthase expression, a marker of mitochondrial content between groups.

Conclusions/Importance: Our results suggest a protective role of SIRT3 overexpression against metabolic dysfunction in offspring exposed to intrauterine GDM and postnatal HFS diet.

Abstract 27

Epigenetics: Is it a Potential Mechanism of Association Between Stress and Albuminuria among Youth with Type 2 Diabetes?

Liat Stitz, Allison Dart, Meaghan Jones, Ola Salama, Nathan Nickel, and Brandy Wicklow

Background/Introduction: Youth with T2D often develop early-onset kidney disease, manifested as albuminuria. Psychological factors like perceived stress may impact progression, although mechanisms remain unclear. This study investigated DNA methylation (DNAm) changes in youth with albuminuria compared to those without, and whether potential changes in DNAm existed in genes known to affect stress functions.

Methods: This cross-sectional study analyzed data from 213 youth with T2D enrolled in the national iCARE cohort study. Kidney injury was assessed by non-orthostatic albuminuria, and perceived stress was measured using the PSS-14 questionnaire. Whole blood DNAm patterns were analyzed. An epigenome-wide association study (EWAS) was conducted to identify differentially methylated sites, with multiple linear regression models. A differentially methylated region (DMR) analysis explored broader DNAm differences across the genome related to kidney injury. A candidate gene analysis compared CpG sites from our study to the EWAS Atlas, with significance assessed using t-tests.

Results: No significant sites were associated with albuminuria based on the EWAS. The top six genes identified through the DMR analysis albeit not statistically significant were: TNXB, TSPAN32, ZNF486, ZNF562, ATP5E, and TNFRSF6B. Some of these genes are linked to mitochondrial function, immune regulation, and stress responses. In the candidate gene analysis, we identified 56 CpG sites with significant differences ($p < 0.05$), with 18 showing stronger significance ($p < 0.01$). However, without multiple testing correction, results should be interpreted with caution.

Conclusion/Importance: Although no significant site-level differences were found, the DMRs suggest potential regions of epigenetic variation that could be associated with both stress and kidney injury in youth with T2D. Given the exploratory nature of these findings and limitations of blood-based DNAm studies, further research is needed to clarify the role of DNAm changes. These findings may help guide future interventions aimed at reducing stress and improving kidney health in this population.

Abstract 28

SIRT3 Deficiency in the Liver is Associated with Mitochondrial Dysfunction and Hepatic Steatosis in Gestational Diabetes

Khushali Trivedi, Bo Xiang, Jewel Paskaruk, Ayesha Saleem & Vernon Dolinsky

Background: Gestational diabetes mellitus (GDM) is the most common transient pregnancy complication that puts mothers and their children at risk for developing type-2 diabetes, obesity, and cardiovascular disease. GDM is characterized by glucose intolerance and insulin resistance. The mechanisms involved are poorly understood. Sirtuin 3 (SIRT3) is a mitochondrial protein deacetylase that regulates energy production in the liver.

Objective: To determine whether deficiency of SIRT3 in the liver is sufficient to induce diabetes during pregnancy.

Methods: Mice with liver-specific-deletion of SIRT3 (SIRT3-LKO) were generated by crossing Sirt3tm1.1Auw mice from Jackson Labs with loxP sites flanking exons 2-3 of the Sirt3 gene with Cre-recombinase mice with an albumin-promoter. SIRT3-LKO mice and Cre-negative controls fed either low fat diet (10% kcal fat) or high fat sucrose diet (45% kcal fat) for 6-weeks before pregnancy and throughout the 3-week mouse pregnancy to induce GDM. Glucose homeostasis was assessed by performing glucose tolerance tests (GTTs) at embryonic day e16 of pregnancy. Pregnant mice were sacrificed at e18.5 and livers were collected for histological visualization using hematoxylin and eosin and Oil Red O. Complex-1 and 2 driven mitochondrial respiration was measured using Agilent Seahorse XFe24 on isolated liver mitochondria.

Results: Genetic deletion of liver SIRT3 is sufficient to induce glucose intolerance ($p<0.01$), hepatic steatosis assessed by quantification of Oil Red O positive area ($p<0.0001$), and significantly reduced mitochondrial basal respiration ($p<0.0001$) in pregnant mice (Two-way ANOVA).

Conclusion: Our findings suggest SIRT3 plays an important role in maintaining adequate mitochondrial function during pregnancy, during an important period when maternal demands for energy production are high. SIRT3 deficiency promotes mitochondrial dysfunction which could contribute to the accumulation of lipids in the liver and glucose intolerance during pregnancy.

Abstract 29

Islet-Derived Extracellular Vesicles – A Potential Biomarker for Amyloid Formation in Type 2 Diabetes and Human Islet Transplants in Type 1 Diabetes

Rushie Tyagi, Janessa Sawatzky, Lucy Marzban

Background: Amyloid formation in pancreatic islets contributes to progressive beta-cell dysfunction/death in Type 2 diabetes (T2D). Amyloid also forms in human islets during pre-transplant culture and post-transplantation in patients with Type 1 diabetes (T1D) which contributes to islet graft failure. Islet amyloid mainly forms by aggregation of human islet amyloid polypeptide (hIAPP; amylin), a peptide hormone normally produced by beta cells. Therapeutic strategies to prevent amyloid-mediated beta-cell death are currently limited by lack of diagnostic tools to assess amyloid formation in patients before irreversible beta-cell damage occurs.

Objective: We examined the role of islet derived extracellular vesicles as a potential biomarker for detection of islet amyloid formation in patients with T2D and transplanted islets in T1D.

Methods: Isolated human islets from cadaveric donors (n=3) were cultured in normal (5.5 mM) glucose (no amyloid) or elevated (11.1 mM) glucose (to form amyloid) for 7 days. EVs were isolated from culture medium, characterized, and their purity was assessed by EV specific markers. (Pro)hIAPP and its aggregates (oligomers) were detected in purified EVs by Western blot and quantified by densitometric analysis. The proportion of beta cells containing oligomer-positive small EVs was assessed by quantitative CD63 (exosome marker) and A11 (oligomer) immunolabelling.

Results: (Pro-hIAPP) forms were detectable in human islets before and after culture in both normal and elevated glucose. Mature hIAPP was the major form detected in EVs from islets cultured in normal glucose while culture with elevated glucose promoted amyloid formation and increased EV content of immature hIAPP forms. Moreover, hIAPP aggregates (oligomers) were detectable in EVs derived from amyloid-forming islets but not amyloid-negative islets.

Conclusion: In summary, these data suggest that EVs released from amyloid-forming human islets contain immature and aggregated forms of hIAPP. Islet-derived EVs may therefore provide a novel biomarker for assessment of amyloid formation in patients with diabetes.

Abstract 30

Metabolism Change with Age across Lake Winnipeg Walleye, Sander vitreus: The Process of Aging on a Wild Model and Its Possible Applications to the Study of Senescence

Lillian Wiens, M. Yusishen, K. Jeffries, D. A. Watkinson, E. Enders, M. Rennie, J. Treberg

Wild animals hold common characteristics with laboratory models. However, they may have other patterns within the internal metabolism not present in common laboratory models. These specific characteristics may be a better illustration of the circumstances that the world outside the laboratory may represent. Many factors influence the internal metabolism of vertebrates, age is one of them. Diabetes is hypothesized to be correlated to accelerated aging. Understanding senescence in the wild maybe of help to fully understand the process of aging in humans. This study determined if age of walleye caught in 2018 can be a predictor of metabolic markers that reflect senescence. Walleye from Lake Winnipeg was sampled for blood, metabolic profiles were determined by using mass spectrometry, MS, and nuclear magnetic resonance, NMR. Telomere length assays were performed on the whole blood of the walleye (data from J. Jeffrey and M. Gaudry). Statistical analyses of the walleye metabolic blood composition were used to understand if metabolic pathways change with chronological age. Results show some molecules found within the whole blood are known to change in concentration during the senescence process in mammals, including humans, and birds. Furthermore, the telomere assays show no senescence patterns on the walleye subpopulation. Lipid like metabolites show a decrease with age within the whole blood. This pattern may represent negligible senescence which is hypothesized to be present in some un-determinate growers. Further study of fish models may clarify if indeed evolutionary processes related to life history traits, like how growth patterns may explain some differences in aging across vertebrates. This may clarify patterns of senescence that are still open ended in mammals as well, and may explain some environmental influences on the process of aging in humans.