abstract 1

Dialectal Behavioural Therapy and Lifestyle Change to Prevent Type 2 Diabetes in Adolescents living with Obesity: A feasibility study

Vibhuti Arya, Jana Slaght, Emily Cameron, Mae Santos, Nicole Brunton, Hannah S. DeVisser, Leslie Roos, Mandy Archibald, Allison Dart, Laurence Katz, Brandy Wicklow, Tracie Afifi, Katherine Morrison, Jonathan McGavock

Introduction: Pediatric obesity affects over 1.5 million Canadian youth and few interventions have proven successful for weight management. Little is known about the efficacy of adding Dialectical Behavioural Therapy (DBT) for improving the adherence to lifestyle change and improving the biopsychosocial health of adolescents living with obesity. The aim of the current pilot trial was to address this gap.

Methodology: We enrolled 6 adolescents 14-17 years of age with a BMI Z-score >1.6 and mild to moderate depressive symptoms to participate with a caregiver in a pilot of a novel DBT and behaviour change intervention. Adolescents received two sessions weekly for 16 weeks focused on (1) developing DBT skills (2) supporting behavioural lifestyle change. The main outcomes were enrollment rates, adherence to the intervention, and retention rates for follow-up measurements. The secondary outcome was changes in quality of life (PedsQL) and depressive symptoms. Adolescents and caregivers also participated in focus group sessions informed by photo elicitation.

Results: Overall, we screened 83 adolescents and enrolled 6 into the pilot. The adherence to the intervention sessions was 83% and 92% for DBT and Lifestyle sessions respectively, and the retention rate was 83%. Several themes identified in the focus group sessions revealed that the invention (1) improved relationships with caregivers, led to new skills for communication and behaviour change and control over emotions. Negative aspects of the intervention included the virtual delivery of DBT, time commitment for the sessions and the lack of personalized skill development.

Conclusion: Delivering DBT skills in conjunction with a behavioural lifestyle intervention for adolescents is possible and was well received but could be improved to personalize skill development.

abstract 2

Trends in gestational diabetes in Manitoba from 1981-2019: A descriptive study

Nicole Brunton, Kevin Friesen, Jennifer Yamamoto, Heather J. Prior, Jonathan McGavock

Background: Incidence of gestational diabetes mellitus (GDM) is increasing globally. However, there is little information describing trends in GDM incidence over time within sub-groups of women that often experience health inequities.

Methods: We performed a registry-based administrative cohort study to describe trends in GDM incidence between 1981-2019, stratified by known risk factors. We compared population trends before and after screening changes (2000 – 2009 vs 2010 – 2019) and stratified by age, urbanicity, and household income using difference-in-differences analyses. Geospatial mapping was used to visualize changes by Winnipeg neighbourhood cluster. In sensitivity analyses, we examined trends in large for gestational age (LGA) births.

Results: GDM incidence increased in Manitoba from 1.3% in 1981 to 8.6% in 2019 with an inflection occurring around 2010 (n = 493,966). From 1981 – 2009 GDM increased by 1.4 percentage points compared to 5.6 between 2010 – 2019. This trend was also observed after stratifying by age, urbanicity, income, and socioeconomic status (SES). Between 2000 and 2019, GDM was consistently highest among women >35 years old and those in the lowest SES category. Difference-in-differences analyses revealed that GDM incidence increased by 1.68 (95%CI: 1.37, 1.99) percentage points more among urban vs rural residents and 3.83 percentage points (95%CI: 3.12, 4.53) among women 35+ years compared to women 18-24 years. Geospatial mapping revealed that GDM incidence increased more in neighbourhoods with the highest proportion of new immigrants. Sensitivity analysis revealed that the proportion of LGA deliveries among mothers with GDM dropped after 2010.

Conclusion: Incidence of GDM is increasing in Manitoba, particularly among those with low SES and higher maternal age. However, this may be partially due to a change in screening practices as evidenced by the upward inflection consistent across strata and concomitant downward trend in LGA infants.

abstract 3

Changes in extracellular vesicle (EV) subtype and secretion markers with replicative senescence in murine pancreatic beta cells

Benjamin Bydak, Marvin Yan, Patience O. Obi, Tamiris F. G. Souza, Samira Seif, Emily Turner-Brannen, Carmen Ching, Adrian West, Adrian Kee Kong Teo, Ayesha Saleem

Introduction: Type 2 diabetes (T2D) is an age-associated disease characterized by a sustained elevated insulin demand, linked to an accumulation of senescent pancreatic β-cells. Senescent cells release pro-inflammatory cytokines, perpetuating increased inflammation, which can be packaged in extracellular vesicles (EVs). EVs are small, membrane-bound nanoparticles that facilitate cellular communication through biological cargo. EVs are characterized as small (sEVs) and medium/large (m/lEVs) EVs. The effect of replicative senescence on the secretion of EVs from pancreatic β-cells is currently unknown.

Methods: Murine pancreatic -cells (MIN6) at low (LP, P22-30) and high (HP, P54-61) passage were grown in EV-depleted media for 48 hrs (N=6, unless otherwise noted). Differential ultracentrifugation and ultrafiltration were used to isolate EVs from conditioned media. EVs were characterized using tunable resistive pulse sensing. Cells were assessed for viability and baseline senescence (beta-galactosidase). Immunoblotting on cell lysates was used to measure proteins related to EV subtypes and secretion.

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. Secretion of m/lEVs was 1.77-fold higher in HP-cells vs. LP-cells (p=0.02). sEV secretion was unchanged between HP vs. LP cells. Average EV size was 9% lower in HP-EVs (113nm) vs. LP-EVs (125nm; p=0.04, N=5), while cell count and viability remained unchanged. Beta-galactosidase staining was ~1.6-fold higher in HP-cells vs. LP-cells (p=0.003, N=3). TSAP6, a regulator of EV secretion, was 68% higher in HP vs. LP cells (p=0.04, N=5), while sEV markers CD9, CD81, TSG101, and ALIX remained unchanged.

Conclusion: β-cells preferentially release small EVs regardless of passage. HP-cells have higher levels of senescence, and show increased m/lEV release vs. LP-cells, concomitant with increased expression of TSAP6. Differences in EV release with replicative senescence may be linked to enhanced EV secretion in pancreatic β-cells. Future work on EV trafficking within HP-cells is warranted.

abstract 4

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

Michael Byun, Samantha Pauls

Background: Chronic inflammation is a driving factor in diseases like obesity and type 2 diabetes. Enhanced glucose metabolism, including via oxidative phosphorylation, may contribute to heightened immune activation. A recent clinical trial showed that supplementation with the omega-3 fatty acid α-linolenic acid (ALA) reduced oxidative phosphorylation rates in circulating monocytes. However, the mechanism remains unknown. 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. The Seahorse XFe24 system was used to approximate catabolic rates (including oxidative phosphorylation and glycolysis) in the presence of either glucose or palmitic acid as metabolic substrate. We also examined mitochondrial reactive oxygen species (ROS) levels using the fluorescent indicator mitoSOX measured by flow cytometry. Finally, gene expression was assessed by reverse-transcription quantitative polymerase chain reaction (RT-qPCR).

Results: ALA significantly reduced mitochondrial ATP production by ~26% and increased glycolytic ATP production by ~50% in the presence of glucose. Unexpectedly, another omega-3 fatty acid, docosahexaenoic acid (DHA) had similar effects. There was no apparent change in fatty acid catabolism. ALA had no effect on ROS while DHA enhanced ROS by ~30%. 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 ALA and DHA by 4- and 13-fold, respectively.

Conclusion: Overall, ALA and DHA both upregulated PDK4 and dampened oxidative phosphorylation rates in our cell culture model. This was accompanied by enhanced ROS in the case of DHA, a sign of mitochondrial stress. This is an important step towards understanding how omega-3 fatty acids may be useful as part of an intervention strategy to prevent or treat chronic metabolic diseases relevant to children and youth.

abstract 5

Urban Trail Infrastructure and Physical Activity Levels: A Systematic Review and Meta-Analysis of Natural Experiments

Isaak Fast, Hannah Steiman de Visser, Nashed, Jack Lötscher, Jonathan McGavock

Background: In response to climate change, cities across Canada are investing over $1B in new cycling infrastructure to support more active transportation. Little empirical evidence exists describing the effectiveness of adding protected cycling trails on changes in cycling or physical activity levels. We hypothesized that areas with new infrastructure would experience increased physical activity and trail use compared to areas without new infrastructure.

Design and 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 an experimental pre-post design that reported physical activity or trail counts for an intervention and control area. The interventions were limited to protected and/or separated bike lanes, including cycle tracks, greenways, and bike lanes with concrete barriers. Our primary outcomes were individual level physical activity and trail use counts. A modified risk of bias tool will be employed to assess the methodological quality of each selected study.

Results: Three independent reviewers screened abstracts from 3936 articles, of which 58 were reviewed for potential full text review. After resolving conflicts, 29 articles describing natural experiments of new cycling infrastructure met eligibility criteria and will be subjected to data extraction and subsequent meta-analysis. We will present the data for population characteristics in both intervention and control areas, such as socioeconomic status, mean age, race, and the percentage of females, as well as outcomes related to physical activity. We will also report the risk of bias for these studies and the degree to which they adhered to TREND reporting guidelines for quasi-experimental studies.

Conclusions: These data will provide the first comprehensive assessment of the impact of new cycling trails on physical activity levels in urban centres.

abstract 6

The Diabetes-Related Gly482Ser Polymorphism Affects PGC-1α Stability and Glucose Metabolism

Maria Galipeau, N Jouvet, E Courty, R Vandenbeek, NA Khan, K Bouyakdan, C Nian, C Iorio, DD Scott, M Faraj, M Oeffinger, FC Lynn, RA Screaton, T Alquier, JL Estall

Introduction: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 is a transcriptional coregulator that plays a major role in controlling metabolism and mitochondrial biogenesis. We showed that a diabetes-associated single nucleotide polymorphism (Gly482Ser, SNPrs8192678) results in decreased protein stability and half-life in liver and beta-cell lines and human induced pluripotent stem cells. We have evidence that degradation of the S482 variant is mediated by phosphorylation at this site (mass spectrometry) by three potential kinases (NEK2, MARK4, and S6KB2).

Method: To study the physiological consequences of this SNP, we generated whole-body homozygous glycine (G/G), serine (S/S) and heterozygous (S/G) mice. Male (N=10-11 per genotype) and female (N=8-9) mice were subjected to standard chow or high fat, high fructose diet (HFHF) for 14 or 24 weeks.

Results: We found that S/S male and female mice on a HFHF diet had decreased caloric intake using metabolic cages without decreased body weight. Male S/S mice secreted more insulin in response to a mixed-meal challenge and had increased glucose uptake in muscle and adipose tissues. We've observed a similar trend in non-diabetic humans, with carriers of the S/S variant secreting more insulin and oxidizing more carbohydrate while those with G/G or G/S variants were oxidizing more fat following the ingestion of a high-fat meal.

Conclusion: These results demonstrate that differences in PGC-1α stability associated with phosphorylation at site 482 may lead to differences in glucose and fat metabolism, which could explain the link between this SNP and metabolic diseases.

abstract 7

Diabetes model of Chronic kidney disease (CKD), rather than nephrectomy-based model, as the choice model?

Abdelaziz Ghanemi, Sylvain Picard, Roth-Visal Ung, Richard Larivière, Mohsen Agharazii, Fabrice Mac-Way

Introduction: With the increase in chronic kidney disease (CKD) cases - including among diabetes and obese patients - and its impacts and consequences at various levels, there is a need to develop animal models to deepen our understating of the underlying pathways in various pathological and physiological contexts to tackle the related challenges.

Methods: In order to study CKD, many animal models have been developed to study the disease, the complications linked to it and also to test/evaluate different therapeutic approaches. Within this presentation, we compare three animal models of CKD including obesity and diabetes animal models. The third model (we use in our lab) is the nephrectomy-based animal model. The different models vary depending on CKD induction method (surgical, diet and chemical, in our examples).

Results: We can develop CKD animal models based - for instance - on obesity/diabetes animal models that - depending on various factors - might develop kidney disease. However, such models will also simultaneously develop obesity/diabetes phenotype along with the kidney disease. This represents a good model that not only leads to CKD but also mimics the pathological process that leads to it (as results of diabetes/obesity complications in our illustrative examples). For the model based on nephrectomy - for example-, it aims to mimic CKD. It exhibits the disease outcome but without mimicking its pathogenesis as it only targets the kidneys through a surgical procedure.

Conclusion: The choice of the suitable model depends on the context (studied pathways, tested therapies, etc.). Other risk factors for CKD (age, diet, inactivity, pathophysiological status, etc.) can be added to the model in order to further optimize it.

abstract 8

A comparison of birth outcomes in the Next Generation cohort based on in utero exposure to diabetes and substance use during pregnancy

Priscilla Irabor, Elizabeth Sellers, Richard LeDuc, Brandy Wicklow

Introduction: Pregnancies complicated by diabetes are at increased risk for adverse birth outcomes. Substance use during pregnancy also contributes to poor birth outcomes. The objective of this project was to examine whether abstaining from substance use during pregnancy can protect infants exposed to diabetes in utero from poorer birth outcomes.

Methodology: The Next Generation cohort includes infants born to mothers with pre-gestational diabetes (T2DM) and gestational diabetes (GDM). Birth outcomes such as birth weight, gestational age, C-sections, admission to NICU, hypoglycemia, and neonatal complications (jaundice, respiratory distress and feeding problems) were obtained from electronic medical records. Substance use (alcohol, tobacco, marijuana, and illicit drugs) during pregnancy was self-reported in maternal pre-natal charts. For birth weight and gestational age, a generalized mixed model was used with diabetes and substance exposure statuses as class variables and an interaction effect included. For the other variables, logistic regression was used.

Results: 215 infants had complete medical records and were included in this analysis. 47.9% were not exposed to substances in utero while 52.1% were exposed to substances in utero. There were no significant associations found between birth outcomes and substance exposure status. Although there were no significant differences between the substance exposure and non exposure groups, it was found that T2DM status increased the risk for C-sections ($p=0.0085$), admission to NICU ($p<0.0001$), hypoglycemia ($p<0.0001$), and neonatal complications ($p=0.0003$) compared to the GDM group.

Conclusion: Although there were no significant associations between substance exposure status and birth outcomes in our cohort, we expect that the abstinence from substances still plays a positive role during pregnancy. As the results show that T2DM was associated with higher rates of adverse birth outcomes compared to GDM, it should inform a more focused approach with research and pre-natal care for women with T2DM.

abstract 9

Geospatial distribution and demographics of Winnipeg neighbourhoods stratified by density of cycling infrastructure: A descriptive study

Nika Klaprat, Kevin Friesen, Heather Prior, Charles Burchill, Jonathan McGavock

Background: Geospatial data for the density of cycling infrastructure in Canadian cities was made available in 2022 through the Canadian Urban Environmental Health Research Consortium (CANUE) database. The aim of this study was to describe the population demographics and neighborhood characteristics associated with the density of cycling infrastructure in Winnipeg.

Methodology: We linked data from the 2016 Canadian Census, the CANUE database and open source data from the City of Winnipeg for this study. The main exposure was the density of high-comfort cycling infrastructure in Winnipeg, quantified with the Canadian Bikeway Comfort and Safety (Can-BICS) Index, which provides a weighted index for the number of kilometers of different types of cycling infrastructure. The main outcomes were neighbourhood-level demographics, socioeconomic status, diversity and aspects of the built environment that support physical activity.

Results: Can-Bics data were available for 18,215 neighbourhoods in Winnipeg. Only 3.8% of Winnipeg residents live in a neighborhood that was classified as high cycling infrastructure density. Compared to neighbourhoods with the lowest density of cycling infrastructure (n= 3893), those with the highest density (826) had 25 times more kilometers of infrastructure (0.4 ± 0.8kms vs 12.2 ± 5.8kms). Neighbourhoods with the highest density of cycling infrastructure were younger (43.4 yrs IQR: 37.7-50.0 yrs vs 47.0 yrs IQR: 43.0-51.1 yrs), had less household income ($47,644 IQR: $42,107-$75,467 vs $85,032 IQR: $70,878-$107,996), a higher proportion of people identifying as a visible minority (43.8% IQR: 27.1-48.3% vs 22% IQR:9.7%-41.7%) and higher walkability (CAN-ALE index: 2.45 IQR: 1.91-3.12 vs 0.10 IQR: -0.19-+0.31).

Discussion: Neighbourhoods with the greatest cycling density in Winnipeg are characterized by lower socio-economic status, higher population diversity and more access to physical activity. These differences influence the design of epidemiological studies of child health outcomes related to exposure to cycling infrastructure.

abstract 10

Preferences for exercise interventions for people living with type 1 diabetes: A discrete choice experiment

Andrea MacIntosh, Dana Greenberg, Marley Greenberg, Nika Klaprat, J Brandt, N Gregoire, S Dostie, D Boutin, C Pow, J Witt, Jonathan McGavock

Background: Regular physical activity is a cornerstone to cardiometabolic health and quality of life for people with type 1 diabetes (T1D). Understanding preferences for exercise training for people with T1D could help increase adherence and retention in future randomized trials.

Methodology: We conducted a discrete choice experiment, distributed as an online survey across Canada between June and August 2021. The survey design included five attributes common to an exercise intervention: exercise type, time of day, and program design, intensity, and duration. Each attribute had three different options or levels within it. Respondents were presented different scenarios involving the attributes and asked to select which levels were most preferable. The survey was co-designed, and pilot tested with patient partners involved on our team.

Results: The final design included 12 questions, each with three alternatives. There were 458 respondents; 280 completed all questions. The median age at diagnosis was 18 years (range: 7-32 years), and 176 (73.3%) were female. Preferences for exercise training programs were a combination of strength and endurance compared to either cardiovascular (-0.104 ± 0.47) or strength (-0.13 ± 0.4) training alone. Individualized exercise was preferred over group-based exercise (-0.12 ± 0.46) and individual exercise with supervision (-0.08 ± 0.46). Medium intensity exercise was preferred over high (-0.17 ± 0.41) or low intensity (0.01 ± 0.41). Sessions 30-60 minutes long were preferred over sessions <30 mins (0.09 ± 0.45) and sessions >60 minutes (-0.24 ± 0.46). Finally, morning and evening sessions were ranked similarly with afternoon sessions the least preferred (-0.09 ± 0.38). Preferences were similar for men and women.

Conclusion: These data could be used to inform future randomized trials of exercise training to increase adherence to an exercise intervention or in clinical practice for diabetes educators supporting individuals living with T1D to adopt a more active lifestyle.

abstract 11

Unraveling the Cellular Sources of Interleukin-1β Production in Amyloid-Forming Human Islets: A Potential Strategy to Enhance Human Islet Graft Survival in Type 1 Diabetes

Danish Malhotra, Lucy Marzban

Purpose: Type 1 diabetes (T1D) is characterized by immune-mediated destruction of pancreatic beta-cells, leading to hyperglycemia. Islet transplantation provides a promising strategy to restore endogenous insulin production and normoglycemia in T1D but is limited by insufficient pancreatic donors and islet loss post-transplantation. Islet amyloid, formed by aggregation of human islet amyloid polypeptide (hIAPP), contributes to loss of beta-cell mass/function in T2D. Amyloid also forms in human islets during pre-transplant culture and post-transplantation, thereby contributing to islet graft failure. We previously showed that amyloid formation promotes islet inflammation and interleukin (IL)-1β production, leading to β-cell upregulation of Fas death receptor and apoptosis. Here, we investigated the potential cellular source(s) of IL-1β production in human islets during ex vivo amyloid formation.

Methods: Isolated human islets (n=5 cadaveric donors) were treated with or without clodronate (to deplete macrophages) and cultured in elevated (11.1 mmol/l) glucose (potentiate amyloid formation) for 7 days. Quantitative immunolabeling was performed on paraffin-embedded islet sections for insulin and each IL-1β, Fas, TUNEL (apoptosis), thioflavin S (amyloid), and CD68 (macrophage marker).

Results: Freshly isolated human islets had low IL-1β immunoreactivity. Islet culture resulted in amyloid formation, which was associated with increased islet IL-1β immunoreactivity (Day 0: 1.0±0.3; Day 7: 8.6±1.7, $p<0.05$), Fas-positive (Day 0: 0.8±0.2%; Day 7: 4.2±1.1%) and TUNEL-positive β-cells (Day 0: 2.2±0.5%; Day 7: 8.1±1.2%), all of which were markedly reduced by amyloid inhibition. Depletion of islet macrophages markedly reduced, but did not completely block, amyloid-induced IL-1β immunoreactivity in islet β-cells (Day 0: 0.9±0.2; Day 7: 7.6±2.3; Day 7+clodronate: 2.9±0.1).

Conclusion: These data suggest that macrophages (mainly) and β-cells are two cellular sources of amyloid-induced IL-1β production in human islets, which leads to Fas-mediated β-cell apoptosis. Blocking islet IL-1β production and/or signaling may provide an effective pharmacological strategy to protect β-cells from amyloid during pre-transplant culture and post-transplantation.

abstract 12

Gestational Diabetes Mellitus Induces Cardiac Dysfunction and Increases the Acetylation of Metabolic Enzymes in the Offspring Heart

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

Background: Exposure to gestational diabetes mellitus (GDM) increases risk of cardiovascular disease in offspring later in life. Previously, our lab found that GDM exposure impaired mitochondrial respiration by the cardiomyocytes of fetal offspring that was associated with cardiac dysfunction. Protein lysine acetylation is a post-translational modification that regulates activity of metabolic enzymes within the mitochondria.

Objectives: To determine how GDM exposure affects protein lysine acetylation in the offspring heart.

Methods: GDM was induced by feeding female mice a high fat sucrose (HFS; 45% fat) diet for 6 weeks prior to mating and throughout pregnancy. Control lean dams were fed a low fat (LF; 10% fat) diet. Offspring from Lean and GDM dams were fed LF and HFS diets. Echocardiography was performed in 15-week-old offspring. Mitochondria were isolated from offspring hearts and acetylated peptides were extracted via immunoprecipitation and quantified by mass spectrometry.

Results: GDM and HFS diet induced cardiac hypertrophy (Lean-LF vs. GDM-HFS $p=0.015$) and diastolic dysfunction (Lean-HFS vs GDM-HFS $p=0.006$) in 15-week aged offspring . GDM exposure differentially altered the acetylation of mitochondrial peptides, which was exacerbated by a postnatal HFS diet (GDM-HFS vs Lean-LF 88 peptides, $p<0.05$). Functional classification revealed prominent representation of acetylated enzymes involved in the TCA cycle, fatty acid oxidation, respiratory electron transport, and mitochondrial biogenesis pathways in the hearts of GDM offspring.

Conclusion: GDM-induced cardiac hypertrophy and diastolic dysfunction in the offspring. These impairments in cardiac structure and functions were found in tandem with increased acetylation of cardiac mitochondrial enzymes regulation energy metabolism. Cardiac mitochondrial enzyme acetylation represents a novel molecular mechanism that contributes to GDM-induced mitochondrial dysfunction and cardiomyopathy in the offspring.

abstract 13

Glucagon-like peptide 1 and 2 double receptor knockout (GLPDRKO) mice have higher post-prandial lipids and glucose in a sex- and meal-dependent manner

Nadya M. Morrow, Chelsea T. Klein, Natasha A. Trzaskalski, Cassandra A. A. Locatelli, Antonio A. Hanson, Serena M. Pulente, Andrew C. Clement, Erin E. Mulvihill

Background: Enteroendocrine cells in the gut epithelium secrete gut hormones in response to nutrients. These hormones include glucagon-like peptide 1 (GLP-1), GLP-2, and glucose-dependent insulinotrophic polypeptide (GIP). GLP-1 and GIP amplify glucose-stimulated insulin secretion from pancreatic islets whereas both GLP-1 and GLP-2 modulate the rate of dietary triglyceride secretion from the intestine into circulation in opposing manners.

Hypothesis: Loss of endogenous GLP-1R and GLP-2R signalling will perturb intestinal lipid metabolism but not glucose homeostasis due to GIPR incretin compensation.

Methods: Male and female 8-week-old Glp1r-/-Glp2r-/- (GLPDRKO) and wild-type-littermate (WT) mice underwent a 24 hour fast and 4-hour refeeding protocol. GLPDRKO and WT mice received a gavage of 1) olive oil to asses lipid tolerance, 2) glucose to assess glucose tolerance, 3) Ensure to assess blood glucose in response to a mixed meal.

Results: Nanostring intestinal mRNA analyses revealed that refed GLPDRKO mice display attenuated expression of genes in antigen presentation and nutrient sensing pathways. Male GLPDRKO mice displayed significantly greater plasma triglyceride levels post-oil compared to WT controls. Lipid tolerance was similar in females. Oral glucose tolerance was similar in male GLPDRKO and WT controls. Surprisingly, female GLPDRKO mice displayed significantly worse glucose tolerance compared to WT controls, and in response to the mixed meal, both male and female GLPDRKO mice displayed higher glucose levels compared to WT controls. As the mice aged (30 weeks), changes in oral lipid tolerance ceased and were not different between genotypes and sexes. Interestingly, oral glucose tolerance was significantly worse in both male and female GLPDRKO compared to WT controls in response to glucose alone and the mixed meal.

Conclusions: Our data reveal an age- and sex-dependent impact of endogenous gut hormone signalling on intestinal lipid metabolism. Additionally, our data suggest that compensatory glucose lowering by GIPR signalling diminishes with age in males.

abstract 14

Maternal Resveratrol (RESV) Supplementation and the Effects on Cardiac Hypertrophy in the Offspring

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 pregnancy condition that is characterized by maternal insulin resistance, glucose intolerance, and hyperglycemia, and impacts maternal and offspring health. Sometimes medications are used, but are associated with the risk of adverse pregnancy outcomes and the long-term effects on the offspring are unknown. Our previous studies observed that GDM-exposed offspring exhibit hypertrophy, mitochondrial dysfunction and impaired calcium flux in rat cardiomyocytes. In this study, we hypothesize that Resveratrol (RESV) in the 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 1.4-fold increased intraventricular septal and left ventricular posterior wall thickness compared to Lean and GDM+RESV offspring (Lean vs. GDM, $p<0.05$)(Lean vs. GDM+RESV, $p<0.05$). Cardiomyocytes isolated form GDM-offspring had approximately 20% lower levels of maximal respiratory capacity compared to Lean and GDM+RESV offspring (Lean vs. GDM, $p<0.05$)(Lean vs. GDM+RESV, $p<0.05$). Furthermore, cardiomyocytes isolated from GDM-offspring exhibited delayed calcium flux cycles compared to Lean offspring.

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. Importantly, maternal RESV supplementation attenuated GDM-induced cardiac hypertrophy in GDM-offspring.

abstract 15

Identification of putative protein targets that mediate enhanced mitochondrial biogenesis in skeletal muscle-derived extracellular vesicles after chronic contractile activity

Patience O. Obi, Tamiris F. G. Souza, René Zahedi, Ying Lao, Richard LeDuc, Joseph W. Gordon, Ayesha Saleem

Introduction: Extracellular vesicles (EVs) are membrane-bound nanoparticles encapsulating biological cargo, and mediate cellular communication. We have previously shown that skeletal muscle-derived EVs released post-chronic contractile activity (CCA) increased mitochondrial biogenesis (MitoB) in murine myoblasts, but the underlying mechanisms are unknown. Here, we investigated the localization of EV protein cargo post-CCA (luminal vs. membrane-bound) and identified putative EV protein targets (using proteomics) that mediate this effect.

Methods: C2C12 murine myotubes were electrically paced (3 hrs/day x 4 days @ 14V, IonOptix). EVs were isolated from conditioned media using differential ultracentrifugation. CCA-EVs were treated with 100 µg/mL Proteinase K (ProK) + 0.1% Triton X-100 (Tr), and then co-cultured with myoblasts for 4 days. Mitochondrial respiration was measured (Seahorse XFe24, Agilent) and data analyzed using Student's t-tests. Liquid chromatography-mass spectrometry (LC-MS/MS) proteomic analysis was performed on control-EVs and CCA-EVs and data analyzed using bioinformatic tools (FunRich, Ingenuity Pathway Analysis, STRING).

Results: CCA-EVs increased basal oxygen consumption rate (OCR) by 52% (p=0.0493, N=6), and maximal OCR by 28% vs. PBS (p=0.0320, N=6). Treatment with ProK + Tr ameliorated this effect (p=0.0292; p=0.0007, N=6). Using LC-MS/MS, a total of 2363 proteins were identified, of which 62 proteins were significantly altered, with 46 increased and 16 decreased in CCA-EVs vs. control-EVs. Pathway analysis identified Cap1-Pfn1-Actn1-Itga6 as significantly altered. These four proteins were also in Top-100 proteins identified in ExoCarta and Vesiclepedia databases. Interestingly, Cap1 and Pfn1 have been reported to mediate mitochondrial function and are both membrane-bound.

Conclusions: Our data show that the CCA-EVs increased basal and maximal OCR, and the effect was mediated by membrane-bound and/or EV corona protein cargo. Additionally, the Cap1-Pfn1-Actn1-Itga6 pathway was significantly increased in CCA-EVs, with all four proteins enriched in EV databases. Our next step will be to validate putative targets to identify proteins regulating the prometabolic effects of CCA-EVs

abstract 16

Exploring the Impact of Anti-CD3 Immunotherapy on Beta Cell Stress in Type 1 Diabetes

Jasmine Pipella, Peter Thompson

Background/Introduction: For the past century, insulin was the sole Federal Drug Administration (FDA) approved type 1 diabetes (T1D) treatment. In November 2022, Teplizumab, an anti-CD3 immunotherapy, received FDA approval to delay symptomatic T1D. However, the mechanism of action of Teplizumab remains unclear. Studies have shown that Teplizumab improves beta cell function, suggesting an impact on beta cell stress. We hypothesize that Teplizumab may reduce beta cell senescence, a novel stress pathway which may adversely affect beta cell function.

Methods: Female non-obese diabetic (NOD) mice received weekly doses of anti-CD3 monoclonal antibody or IgG control treatment from weeks 9-13. Intraperitoneal glucose tolerance tests (IPGTTs) were conducted to assess beta cell function prior to isolation of whole pancreas or pancreatic islets. Senescent beta cell frequency and gene/protein expression were evaluated using immunohistochemistry (IHC), reverse transcriptase quantitative polymerase chain reaction (RT-qPCR), and Luminex, respectively. The anti-CD3 treatment was validated using flow cytometry on splenocytes.

Results: Significant modulation of total CD3+ T cells ($p=0.0020$) and changes in antigen-specific T cells were observed in the anti-CD3 treated mice compared to the IgG controls. IHC revealed a significant increase in insulin+ stained area ($p=0.0104$), reduced insulitis and a significant decrease in senescent beta cells ($p=0<0.0001$) in the anti-CD3 mice but no significant difference in markers of beta cell identity or unfolded protein response pathways.

Conclusions: Our findings in NOD mice suggest a novel mechanism for the action of anti-CD3 at the late pre-diabetic stage. Specifically, the modulation of cytotoxic T cells with anti-CD3 may slow disease progression in part by limiting the accumulation of senescent beta cells. These findings raise questions about the potential interplay between cytotoxic T cell depletion, beta cell function, and senescence in T1D while offering exciting avenues to investigate the relationship between these factors.

abstract 17

Investigating the Relationship Between Insulin Synthesis and Senescence in Type 1 Diabetes

Camille Prefontaine, Gabriel Brawerman, Peter Thompson

Introduction: Recent work has shown that a subpopulation of β-cells becomes senescent during the progression of type 1 diabetes. Notably, islets induced to be senescent show a significant decrease in total insulin content. To date, the specific cause of lowered insulin content in senescent beta cells remains unknown. Transcriptomics experiments have revealed that senescent islets do not show changes in INS coding RNA content. This suggests that the decrease in insulin occurs at the translational or post-translational level rather than at the transcriptional level. I hypothesize the decrease in insulin content results from the decreased expression of pro-insulin processing enzymes in human islet beta cells.

Methods: Human donor islets (n= 4 donors) and EndoC- βH5 cells were treated with DNA damaging agent Bleomycin to induce senescence. The cells were then harvested and lysed to evaluate the protein expression of three proteinases responsible for converting proinsulin into insulin and c-peptide in addition to an inhibitor. Differential protein expression of PC1/3, PC2, CPE and a PC1/3 inhibitor ProSAAS were measured by Western blot assay and measured by film densitometry.

Results: The proteinase enzyme increased when senescence was induced, although there was donor variation. Younger donors had a significant increase in PC1/3 and CPE. This phenotype was not observed in EndoC- βH5 cells. These cells had a significant decrease in PC1/3 ($p = 0.0057$), with the other two enzymes showing no change in expression. Regarding the inhibitor ProSAAS, all donors tested showed upregulation of this protein. However, the EndoC- βH5 cells had no observable change in expression.

Main findings: While further sampling is needed, these results do not support the initial hypothesis. Rather they show that in senescence many of the enzymes involved in proinsulin processing are upregulated. Further, an inhibitor is upregulated. This inhibition early in the insulin production pathway could have significant effects on insulin synthesis in senescent β-cells.

abstract 18

GDF15 neutralization as a potential drug target in human senescent beta cells

Nayara Rampazzo Morelli, Peter Thompson

Background/Introduction: Beta cell dysfunction can be activated by different stress pathways during Type 1 Diabetes (T1D) development, such as DNA damage leading to senescence, a type of cell growth arrest. Senescent beta cells show a prosurvival phenotype as well as are characterized by the senescence-associated secretory phenotype (SASP), which includes cytokines, chemokines, and growth factors. A previous work from our lab described a panel of 5 SASP factors secreted by senescent human beta cell models, including donor islets and EndoC-βH5 cell line. Among all 5 SASP factors analysed, the secretion of GDF15 (a growth factor) was highlighted for being significantly increased in both senescent human islets and EndoC-βH5 cells. We hypothesized that GDF15 signaling is contributing with the prosurvival phenotype in senescent human beta cells.

Methods: We tested the neutralizing antibody for GDF15 and its receptor GFR alpha-like (GFRAL) using a GDF15:GFRAL [Biotinylated] Inhibitor Screening Assay Kit. The doses of 10, 5, and 2 µg/mL for GDF15 antibody were selected to be tested in EndoC-βH5 cells, using a non-specific IgG antibody (10 µg/mL) as a control. Previously, cells were treated with bleomycin (35 µM) to induce senescence or vehicle (DMSO). An ELISA assay was performed using conditioned media to measure the GDF15 levels.

Results: We were able to validate the neutralizing effects from both GDF15 and GFRAL antibodies with the screening kit. The experiment with EndoC-βH5 cells also confirmed the presence and neutralization of GDF15 with all antibody doses tested in the senescent cells, while the levels of GDF15 in the vehicle cells were not detected.

Conclusions/Importance: Our preliminary data strongly suggests the secretion of GDF15 by senescent human beta cells and its neutralization as a potential drug target. However, the understanding about how this SASP factor contributes with senescent beta cells survival is lacking.

abstract 19

In-utero Exposure to Maternal Diabetes and DNA Methylation Alterations in the Next Generation Birth Cohort

Ola E. Salama, Yash Rawal, Priscilla Irabor, Christy Pylypjuk, Elizabeth Sellers, Brandy Wicklow, Meaghan J. Jones

Introduction: The incidence of type 2 diabetes (T2D) in youth is increasing and in-utero exposure to maternal diabetes is a known risk factor, with higher risk associated with pregestational T2D exposure compared to GDM exposure. We hypothesize that this occurs through DNA methylation (DNAm) changes induced by in-utero exposure to maternal diabetes that predispose offspring to hyperglycemia. This study presents an epigenome-wide investigation of DNAm alterations associated with in-utero exposure to maternal diabetes, and specifically compares maternal pregestational T2D and gestational diabetes mellitus (GDM), to determine whether the timing of prenatal diabetes exposure alters DNAm differently.

Methods: We performed an epigenome-wide analysis on cord blood from 50 newborns exposed to GDM, 72 exposed to pregestational T2D and 28 unexposed to diabetes in-utero from the Next Generation Birth Cohort.

Results: We identified 19 differentially methylated sites associated with exposure to GDM, 46 sites associated with exposure to T2D, and 11 sites associated with exposure to both GDM and T2D (adjusted p-value < 0.05 and effect size estimate > 0.01). DNAm changes associated with both T2D and GDM in the HACD4 gene were observed only in males. One site at SKAP1 and one site on an unannotated gene were previously associated with obesity. While we did not identify specific CpG sites previously associated with having diabetes, we identified a novel CpG site in the PTPRN2 gene, a gene previously associated with DNAm differences associated with diabetes.

Conclusion: Our findings suggest that in-utero exposure to maternal diabetes is associated with DNAm alterations in offspring. Moreover, the timing of maternal diabetes in-utero exposure (GDM or T2D) produces different DNAm patterns, suggesting that the widow of exposure to maternal diabetes produces different molecular modifications and may explain, at least in part, the difference in risk for youth onset T2D in offspring.

abstract 20

Investigating the Effect of the HNF-1a G319S Variant on Islet Function after Prolonged Fasting

Manuel Sebastian, Taylor S. Morriseau, Kristin Hunt, Christine A. Doucette

Introduction: The HNF-1a G319S variant found in Anishininew communities in central Canada is strongly associated with type 2 diabetes (T2D). However, T2D only recently emerged in these communities while the variant has been present for generations longer. We hypothesized that G319S confers resilience to prolonged fasting and diets associated with a traditional off the land lifestyle, while it interacts negatively with a modern diet, driving metabolic dysfunction.

Methods: The G319S variant was knocked in to C57BL6 mice. Mice were weaned onto a standard chow diet, a high-fat and low-carbohydrate (HFLC) diet (reflective of a traditional diet), or a high-fat and high-carbohydrate (HFHC) diet, reflective of a modern diet. At 3 months, mice were fasted for 24 hours, glucose tolerance was assessed, and tissues collected for various measurements.

Results: After fasting, chow fed G319S mice showed reduced insulin content and Ins2 gene expression. Increased Ldha and Cpt1a gene expression was also observed in islets, along with an increased percentage of immature insulin granules. Glucose tolerance was impaired in S-allele mice on a HF/HC diet but not impaired when these mice consumed a traditional HF/LC diet.

Conclusion: Diet interacted with the G319S variant to influence the metabolic response to fasting such that the variant promoted metabolic resilience to a diet high in fat, similar to a traditional off-the-land diet. Additionally, when carbohydrate content was elevated, the variant associates with poorer metabolic health, which may contribute to higher rates of diabetes in carriers of the variant S-allele. Future studies will investigate the mechanisms involved in these interactions.

abstract 21

Prevalence of severe hypoglycemia in type 2 diabetes in pregnancy

Sarah Sigurdson, Reza Behdani, Reid Whitlock, Jennifer M. Yamamoto

Background: Tight glycemic targets are important to reduce the risk of pregnancy complications. However, there are limited data examining the risk of severe hypoglycemia in people with type 2 diabetes in pregnancy. Our aim was to investigate the risk of severe hypoglycemia among pregnant individuals with insulin-treated type 2 diabetes as well as identify possible risk factors.

Methods: This was a retrospective cohort study in Manitoba between Apr2007-Dec2018. Pregnant individuals aged ≥18 years with type 2 diabetes who received an insulin prescription during the study period were eligible for inclusion. Our primary outcome was severe hypoglycemia defined using a published case definition which included an emergency department visit or hospitalization for hypoglycemia, or a plasma glucose level of <3.0 mmol/L. We used Cox proportional hazard regression models to analyze the time to event until the first occurrence of severe hypoglycemia.

Results: A total of 46,099 subjects with an insulin prescription were identified during the study period. Of those individuals, 2,949 subjects had an insulin prescription within 1 year prior to birth and 90 days after birth. After exclusions, 2,217 patients were included in the analysis. The number of person-years on insulin was 3,416 (4.98 per 1,000 person-years). Within the pregnancy cohort, 17 hypoglycemic events occurred. Of these, 52.9% (n=9) of hypoglycemic events took place during the 2nd or 3rd trimester, while the remaining 47.1% (n=8) took place after birth. For those that had hypoglycemia, their mean diabetes duration was 2.4 years (adjusted HR =1.13 [95% CI: 1.04-1.22] per year of diabetes duration).

Conclusions: Reassuringly, severe hypoglycemia was uncommon in this cohort of people with type 2 diabetes on insulin in pregnancy. Future studies with larger sample sizes are required to further examine risk factors for severe hypoglycemia during pregnancy.

abstract 22

Epigenetics: A Potential Mechanism of Association Between Stress and Albuminuria among Adolescents with Type 2 Diabetes

Liat Stitz, Allison Dart, Meaghan Jones, Nathan Nickel, Brandy Wicklow

Background: Type 2 Diabetes (T2D) is increasing, and in Manitoba particularly affects Indigenous peoples. Adolescents with T2D typically experience complications such as renal disease at earlier stages in their disease. Reported stress is higher in adolescents with chronic disease which is expected to play a role in overall health and disease outcomes. Our objective is to explore the relationship between chronic stress and renal outcomes and examine epigenetics as one potential mechanism by which this occurs.

Methods: This is a cross sectional study using data from the longitudinal iCARE cohort examining differences in DNA methylation patterns between adolescents with and without albuminuria. We will interrogate any methylation differences to determine if they are within loci associated with the physiologic stress response and/or albuminuria. Finally, we will analyze whether there are additive effects on DNA methylation between T2D and stress on renal outcomes. Epigenome-wide association studies and gene ontology will be used to model the relationship between stress and albuminuria.

Results: There are 222 adolescents with T2D and epigenetic data collected included in our analyses. The average age at entry into the study is 15 (±2.3) years. 219 of the participants have completed the PSS14 questionnaire, and 53.88% meet the cut off used to indicate stress. It is anticipated that results will show associations between DNA methylation patterns of both stress and albuminuria. This will show that limiting perceived stress must be prioritized. By understanding the severe outcomes associated with stress, the goal is to make evidence-based recommendations to limit stress.

Conclusion: Improving the renal outcomes of adolescents with T2D in Manitoba, the majority of whom are Indigenous and face more severe renal outcomes, is critical in improving the overall health of our population. A better understanding of the mechanism relating stress to negative renal outcomes, is crucial in this endeavour.

abstract 23

SIRT3 Deficiency in the Liver and Mitochondrial Dysfunction in Gestational Diabetes

Khushali Trivedi, Bo Xiang, Ayesha Saleem, Vernon Dolinsky

Introduction: 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 later in life. GDM is characterized by glucose intolerance and insulin resistance and the mechanisms involved are poorly understood. Sirtuin 3 (SIRT3) is an important mitochondrial protein deacetylase that regulates energy production in the liver. The objective of this study is 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 were fed either a low-fat diet (LF; 10% kcal fat) or a high-fat and sucrose diet (HFS; 45% kcal fat) for 6 weeks before pregnancy and throughout the 3-week mouse pregnancy to induce GDM. Glucose tolerance tests were performed at e17. Pregnant mice were sacrificed at e18.5 and livers collected. Hematoxylin-eosin staining of liver sections was performed to assess lipid accumulation. Liver mitochondria were isolated at e18.5 and mitochondrial respiration was measured using Agilent-Seahorse XFe24 to assess mitochondrial function.

Results: Compared to Cre-negative littermate controls, genetic deletion of SIRT3 in the liver was sufficient to induce glucose intolerance in pregnant mice ($p<0.05$). Histological visualization revealed hepatic steatosis in SIRT3-LKO mice. Liver mitochondria from SIRT3-LKO mice had a 30% reduction of basal respiration compared to controls.

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

abstract 24

A potential cellular mechanism for amyloid-induced beta-cell death in human islets – Implications in pre-transplant human islet culture

Rushie Tyagi, Danish Malhotra, Lucy Marzban

Purpose: In type 1 diabetes (T1D), immune-mediated beta-cell destruction leads to reduced islet beta-cell mass, elevated blood glucose, and life-time insulin therapy. Islet transplantation as a means of beta-cell replacement is a promising treatment approach but is currently limited by loss of islets during pre-transplant culture period and post-transplantation. Formation of toxic protein aggregates named amyloid in human islets due to fibrillogenesis of the beta-cell hormone, human islet amyloid polypeptide (hIAPP), contributes to islet loss during pre-transplant culture and post-transplantation, potentially leading to islet graft failure. The cellular mechanisms by which amyloid destroys beta cells are unclear. We examined the potential role of islet-derived extracellular vesicles (EVs) in amyloid-induced beta-cell death.

Methods: Isolated human islets (n=4 cadaveric donors) were cultured in normal (5.5 mM, no amyloid) or elevated (11.1 mM; form amyloid) glucose for 7 days. EVs were isolated from islet culture medium and their purity was assessed by EV specific markers. Freshly isolated human islets were then cultured for 3 or 7 days without or with EVs purified from control or amyloid-forming human islets (conditioned medium). Amyloid formation and beta-cell death were assessed by quantitative immunolabelling for insulin and thioflavin S (amyloid) or insulin and TUNEL (apoptosis), respectively.

Results: Human islets cultured with the conditioned medium containing EVs isolated from amyloid-forming islets (elevated glucose) had higher amyloid formation than islets cultured with isolated EVs from non-amyloid forming islets (normal glucose) or non-treated islets. Increased amyloid formation in human islets cultured with conditioned medium containing EVs from amyloid-forming islets closely correlated with the increased number of TUNEL-positive beta-cells in those islets.

Conclusion: These data suggest that EVs released from non-healthy amyloid-forming human islets can promote amyloid formation and beta-cell death in healthy islets. Thus, modulating islet-derived EVs may provide a potential therapeutic strategy to improve beta-cell survival during pre-transplant culture period.

abstract 25

Investigating immune surveillance of senescent beta cells in type 1 diabetes

Jay Wieler, Peter J. Thompson

Background: Type 1 Diabetes (T1D) results from autoimmunity leading to destruction of insulin producing beta cells. As an alternative to cell death, some beta cells adopt a senescent state during development of T1D.. Senescence is a programmed growth arrest involved in several physiological and pathological processes and occurs at higher rates in beta cells during T1D. Senescent cells develop an Senescence Associated Secretory Phenotype (SASP), which promotes immune surveillance and clearance. However, whether senescent beta cells undergo immune surveillance and clearance during T1D is not known. Here, we investigated whether a particular innate immune cell, Natural Killer (NK) cells, may contribute to surveillance of senescent beta cells in T1D.

Methods: Immunohistochemistry of NK cells and senescent beta cells was performed on pancreas tissue from the nonobese diabetic (NOD) T1D mouse model, alongside manipulations to activate/expand NK cells. In vitro cell killing assays were performed using a human NK cell line and a human beta cell model for senescence.

Results: We confirmed that NK cells are present in the pancreas of NOD mice. Treatment of NOD mice with a synthetic NK-activating ligand PolyI:C expanded NK cells, slowed disease progression and showed a trend towards reduced senescent beta cells as compared with vehicle control mice. Using a well-studied primary human fibroblast cell line (IMR-90), and a human beta cell line (EndoC), we explored interactions between senescent human beta cells and a human NK cell line NK-92. Unlike senescent IMR-90 cells, senescent EndoC beta cells were resistant to killing by NK-92 cells and secreted high concentrations of cytokine GDF15, which may interfere with NK-92 killing activity. In conclusion, our results provide evidence that NK cells contribute to clearance of senescent beta cells in NOD mice. Additional studies are required to determine the direct cytotoxic activity of NK cells towards senescent human beta cells.

abstract 26

The Peripheral and Central Cross Talk Mediated by Adipose MIF—A Novel Mechanism of Hyperphagia Following Olanzapine Treatment

Xi Chen, Yiheng Huang, Liujun Chen, Yadan Qi, Lisha Li, Pingyi Gao, Donghong Cui, Dake Qi

Introduction: Olanzapine is one of the first-line antipsychotic medications but it usually induces metabolic side effects, characterized by obesity. We previously indicated that olanzapine leads to hyperphagia through the effects of a proinflammatory cytokine, macrophage migration inhibitory factor (MIF) in the hypothalamus. However, whether olanzapine's upregulated peripheral MIF could affect the central mechanism is currently unknown.

Method: We used cell and animal models to investigate the mechanisms of MIF expression and release from adipose tissue following olanzapine treatment, and examine how peripheral MIF regulates hyperphagia.

Results: Our present study found that olanzapine increases ectopic expression of dopamine receptor 1 (DRD1) in adipocytes which relatively upregulates MIF expression and release through a PKA/CREB signaling pathway. In global MIF knockout mice, MIF accumulation appears in the hypothalamus following peripheral injection of recombinant MIF proteins, suggesting that peripheral MIF indeed travels to the hypothalamus. Mif Lung Tg mice (MIF overexpression in the lung) with increased circulating MIF levels had hyperphagia and activation of the CaMKKβ/AMPK/AgRP signaling pathway in the hypothalamus.

Conclusion: Overall, our study for the first time proved that adipose-derived MIF is a crucial factor in regulating metabolic side effects induced by olanzapine.