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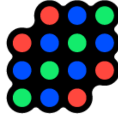

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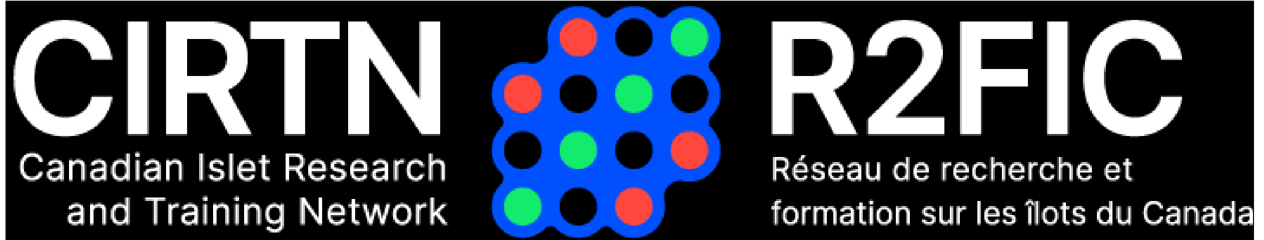

National Meeting
October 28-30, 2023

Institut de Recherches Clinique de Montreal (IRCM)

MONTREAL
QUEBEC

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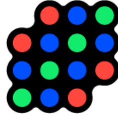

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The meeting's Schedule of Events can be found at

islets.ca/national-meeting-2023/

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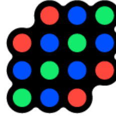

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Welcome! *From the CIRTN-R2FIC Leadership Group*

Welcome to the second-annual Canadian Islet Research and Training Network – Reseau de Recherche et Formation sur les Ilots du Canada (CIRTN-R2FIC) National Meeting! The past three years have seen significant growth of our network (which now has over 250 members), and the National Meeting is an exciting in-person opportunity for us to come together as a community to connect and share the innovative work that we are all doing.

We are absolutely delighted to see so many established and budding islet biologists make the trek to Montreal from all over Canada. This year, we have 80 attendees from 14 universities/research institutes and five provinces. Over the next three days, you can look forward to a fascinating keynote lecture from the esteemed Dr. Carmella Evans-Molina (Indiana University), dozens of outstanding trainee presentations, three professional development workshops, many fun networking opportunities, and leading science across the spectrum of islet and diabetes research and care.

Events like this are made possible by the contributions and hard work of many people and organizations. In particular, we would like to thank our meeting sponsors (pg. 7) for supporting the meeting and allowing us to provide additional activities and perks. We would also like to thank the IRCM and its staff, our generous meeting hosts, for providing us with wonderful service and a beautiful venue.

So, HAVE FUN, LEARN LOTS & most importantly, MAKE CONNECTIONS!

Sincerely,

The CIRTN-R2FIC Leadership Group:

Patrick MacDonald

Jenny Bruin

Jennifer Estall

Bruce Verchere

Jon Rocheleau

Christine Doucette

CIRTN R2FIC
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Who we are, our mission and vision...

The Canadian Islet Research and Training Network (CIRTN-R2FIC) was established in 2020 by joint contributions from the University of Alberta, University of British Columbia, University of Manitoba, Université de Montréal, Insitut de reserches cliniques de Montréal, and the University of Toronto. CIRTN currently includes over 50 laboratories and more than 250 associated researchers and staff engaged in the study of the pancreatic islets of Langerhans. CIRTN-R2FIC seeks to build upon Canada's reputation for excellence in islet biology research by facilitating the exchange of information and ideas with in-person and virtual scientific meetings, by enhancing mentorship and trainee career development, and by promoting engagement and collaboration amongst islet researchers in Canada and worldwide. We will develop approaches to knowledge mobilization and engagement across Canada in a manner that fosters inclusivity.

LEADERSHIP GROUP:

Patrick MacDonald (outgoing Chair), Jen Estall (incoming Chair), Jenny Bruin (vice-Chair), Bruce Verchere, Jonathan Rocheleau, Christine Doucette

STRATEGIC PLANNING COMMITTEE

Vincent Poitout (outgoing Chair), Jim Johnson (incoming co-Chair), Erin Mulvihill (incoming co-Chair), Francis Lynn, Elizabeth Rideout, Peter Thompson, Noa Gang

MENTORSHIP COMMITTEE:

Mathieu Ferron (Chair), Savita Dhanvantari (incoming vice-Chair), Jenny Bruin, Andrew Pepper, Emily Hoffman

TRAINEE WORKING GROUP:

Hui Huang (Chair), Emily Hoffman (Secretary), Amanda Oakie, Siyi He, Jana Palaniyandi, Angela Ching, Cassie Locatelli, Jane Velghe, Zuraya Elisa Angeles Olvera, Clara Goubault, Liam Hall

EQUITY, DIVERSITY, AND INCLUSION COMMITTEE:

Elizabeth Rideout & Gareth Lim (Co-Chairs), Chris Peacocke, Tina Dafoe, Cara Ellis, Jasmine Maghera, Jana Palaniyandi

NATIONAL MEETING PLANNING COMMITTEE:

Christine Doucette (Chair), Chris Peacocke, Jen Estall, Vijaya Madhoo
Amanda Oakie, Nerea Cuesta Gomez, Carol Huang, Dan Luciani, Ekaterina Filatov, Erin Mulvihill, Gareth Lim, Jenny Bruin, Jim Johnson, Kevin Guo, Mathieu Ferron, Siyi He, Vincent Poitout

NATIONAL ISLET BIOLOGY COURSE:

Coordinators: Rob Screaton & Erin Mulvihill | Administrative Support from Carrie Harber and Chris Peacocke

A bit about our Keynote Speaker...

Dr. Carmella Evans-Molina, MD, PhD.

Dr. Evans-Molina is a Physician-Scientist at the Indiana University School of Medicine with subspecialty training in endocrinology, diabetes, and metabolism. The goal of her basic science research program is to define the molecular and inflammatory etiologies of β cell dysfunction in type 1 and type 2 diabetes with a thematic focus on the role and regulation of calcium within the β cell secretory pathway. To support this work, her lab has developed expertise in cadre of methods focused on quantifying and imaging β cell health and function, including measurement of insulin production and secretion, assessment of β cell calcium regulation at the organelle level, assessment of β cell specific transcriptional networks and stress signaling pathways, and whole-animal based physiologic investigation. This work is complimented by her role as Director of the NIH-funded Indiana Diabetes Research Center Islet and Physiology Core. In addition to this basic research focus, Dr. Evans-Molina has a translational/clinical interest in defining the natural history of β cell loss in Type 1 diabetes and “omics” approaches to identify novel serum biomarkers of early β cell stress in pre-symptomatic Type 1 diabetes. This work has been facilitated through her involvement in the Type 1 Diabetes TrialNet Network, where Dr. Evans-Molina serves as Chair of the β Cell and Metabolism Working Group, the Type 1 Diabetes Exchange Registry, and the Human Islet Research Network, where she serves as Chair of the Translational Working Group.

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Mentorship Awards

As a research and training network, each year we recognize two exceptional CIRTN-R2FIC members, one PI and one non-PI, at our National Meeting for their above-and-beyond contributions to mentorship, leadership, or training within the network. All CIRTN-R2FIC members are invited to submit nominations for these awards, and every year we have many deserving nominees to select from. Every nominee has a demonstrated track record of exemplary mentorship, including, fostering supportive research and learning environments, supporting mentee skill development, encouraging intellectual, creative and professional growth, facilitating mentee opportunities and successes and a demonstrated commitment to the principals of equity, diversity and inclusivity in our research community.

Previous winners of these awards:

- 2022 Mentorship Award: Patrick MacDonald
- 2022 Leadership Award: Noa Gang

The 2023 Mentorship Award goes to…. **Dr. Erin Mulvihill**

Dr. Erin Mulvihill is a scientist at the University of Ottawa Heart Institute and an Associate Professor at the University of Ottawa in the Department of Biochemistry, Microbiology and Immunology. Dr. Mulvihill developed expertise in lipid biology and lipoprotein metabolism completing her Ph.D. in Biochemistry at the University of Western Ontario in the lab of Dr. Murray Huff and gut hormones, diabetes and cardiovascular disease while pursuing post-doctoral research under the mentorship of Dr. Daniel Drucker at the Lunenfeld-Tanenbaum Research Institute. The Mulvihill Lab studies the actions and regulation of the bioactivity of islet and gut hormones, glucose and lipid metabolism in the context of obesity, type 2 diabetes and cardiovascular disease. Dr. Mulvihill's contributions to CIRTN have been pivotal to the network's success and include co-leading the CIRTN National Islet Biology course, developing and implementing various career development and training workshops and being a mentor to NSERC-CREATE trainees. We look forward to celebrating these contributions during Dr. Mulvihill's Mentorship Award Lecture on Saturday evening!

The **Leadership Award…** will be announced and recognized in-person at the Meeting (Saturday Oct 28th).

The 2023 Leadership Award is generously sponsored by the CRCHUM.

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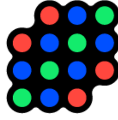

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Thank you to our generous 2023 Sponsors!

CIHR IRSC | Institute of Nutrition, Metabolism and Diabetes
Institut de la nutrition, du métabolisme et du diabète

BC DIABETES
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SCIENTIFIC ABSTRACTS *(by session)*

Session 1: Wait what?! There's more to life than the beta cell??!!

Ketogenic Diet Intervention Elevates Incretins and Impairs Glucagon in Obese Mice

CASSANDRA A.A. LOCATELLI and ERIN E. MULVIHILL

Ottawa Heart Institute, University of Ottawa, Department of Biochemistry, Microbiology and Immunology

Every day 480 Canadians are diagnosed with diabetes and diet intervention is the first-line management tool. In 2020, the extremely high fat, ketogenic diet (KD) was approved by Diabetes Canada for the management of type 2 diabetes (T2D). In short term studies, individuals with T2D lost weight, improved glucose control, and reduced or discontinued T2D medications when they adhered to a KD. However, it is unclear if the nutrient substitution of lipids for carbohydrates has a direct impact on hormonal control of metabolism. To model ketogenic diet intervention, mice were fed high-fat, high-cholesterol (HFHC) diet for 10 weeks to induce metabolic dysregulation. Then mice either continued on HFHC, or switched to standard-lab-chow, or KD, for a further 12 weeks. Male mice had no differences in body weight; however, KD-fed females reduced weight and maintained a lower body weight over time. During a glucose tolerance test, glycemia over time was not different in males, but female KD-fed mice, despite adipose tissue loss, had elevated glucose over time. Interestingly, incretin levels were elevated in KD-fed mice, but insulin secretion was not enhanced. To investigate this further, islets were isolated and underwent dynamic perifusion. No difference in insulin secretion was detected in response to glucose or incretins. Interestingly, KD-fed mice had lower glucagon before and after glucose gavage and glucagon secretion at low glucose was diminished in perifusion. Together, KD intervention did not enhance, or diminished, islet hormone secretion in obese mice.

Connecting specific central GLP-1 receptors functionally with glucose homeostasis and energy balance

ISHNOOR SINGH [1], L. WANG [2], Z. PANG [2,3], D. D. BELSHAM [1,4], M. B. WHEELER [1,5]

[1]Univ. of Toronto, Toronto, ON, Canada; [2]Rutgers Univ., New Brunswick, NJ; [3]The Child Hlth. Inst. of New Jersey, Robert Wood Johnson Med. Sch., New Brunswick, NJ; [4]Departments of Obstetrics/Gynecology and Med., Univ. of Toronto, Toronto, ON, Canada; [5]Div. of Advanced Diagnostics, Metabolism, Toronto Gen. Res. Inst., Toronto, ON, Canada

Central nervous system(CNS) control of metabolism plays a pivotal role in maintaining energy and glucose homeostasis. In the brain, Glucagon-like peptide 1(GLP-1), encoded by the proglucagon 'Gcg' gene, produced in a distinct population of neurons in the nucleus tractus solitarius (NTS), has been shown to regulate feeding behavior leading to the suppression of appetite. However, neuronal networks that mediate endogenous GLP-1 action in the CNS on feeding and blood glucose are not well understood. This is mainly due to the presence of diverse neuronal subtypes and complex central neuronal connectivity. We systematically analyzed the distribution of GLP-1R neurons and axonal projections of NTS^{Gcg} proglucagon expressing neurons in the mouse brain. GLP-1R neurons were found to be broadly distributed in the brain and specific forebrain regions, particularly the hypothalamus, including the arcuate nucleus of the hypothalamus (ARC), received dense NTS^{Gcg} neuronal projections. In addition, we validated the neuronal map of CNS connecting to the pancreas and identified the GLP-1R neurons which might play a role in pancreatic function. We further used this data to study sexual dimorphism in the central innervation of the pancreas. Our result confirmed sex-related difference in the pancreatic innervation. For this reason, the impact of GLP-1 signaling in the ARC, a brain region known to regulate energy homeostasis and feeding behavior was examined. Using a chemogenetic approach to activate the ARC GLP-1R neurons by using Cre-dependent hM3Dq AAV, under the conditions studied we established that activation of the ARC GLP-1R neurons significantly suppressed food intake with a mild improvement in the glucose tolerance. These results highlight the importance of central GLP- 1 signaling within the ARC that express GLP-1R which upon activation, regulates energy homeostasis.

Transcriptional Identification of Human Islet-Resident Immune Cells

Jane Velghe [1,2], Francis Lynn [2,3,4], C. Bruce Verchere [1,2,4,5]

[1] Department of Pathology and Laboratory Medicine, The University of British Columbia, Vancouver, BC; [2] Diabetes Research Group, BC Children's Hospital Research Institute, Vancouver, BC; [3] School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada; [4] Department of Surgery, The University of British Columbia, Vancouver, BC; [5] Centre for Molecular Medicine and Therapeutics, The University of British Columbia, Vancouver, BC

Macrophages are innate immune cells that can shift their phenotype based on their surrounding environment. In humans, pancreatic islets are estimated to contain ~6-10 macrophages; however, islet-resident macrophages play important roles in islet development, remodelling, angiogenesis, and post-injury proliferation of beta-cells, suggesting that islet macrophages are potential targets for diabetes therapy. Mouse studies suggest that islet macrophages express regenerative factors in response to beta-cell death, obesity, or in other states of islet remodelling. To better understand the role macrophages and other immune cells play in islet regeneration and function, we utilize single cell RNA sequencing (scRNAseq) on human islets from lean, obese, and type 2 diabetic donors. We magnetically isolate CD45+ immune cells from other islet cells, and then prepare both cell populations individually for sequencing to obtain sufficient read-depth of rare islet-resident immune cells. Myeloid cells dominate the dataset, with mast cells making up most immune cells in the islet. Preliminary cluster analysis reveals islet macrophage heterogeneity in islets from

healthy donors, with both pro-inflammatory and regenerative populations. In addition to macrophages, mast cells as well as T, NK, and B lymphocytes were detected. Beyond immune cells, endothelial and mesenchymal cells are also present. Our ability to study quality human islet tissue using high-throughput scRNAseq promises a better understanding of pathways that promote β-cell function in humans. These data provide new insight into the transcriptomic profile of human islet-resident immune cells in both health and disease.

Langerhans Islets Transplantation in the Eye to Promote Neuronal Regeneration during Glaucoma.

SANA EL HAJJI [1,2] , CLARA GOUBAULT [1,3] , YUKIHIRO SHIGA [1,2] , LAURA REININGER [1] , MÉLANIE ETHIER - , VINCENT POITOUT [1,4] , ADRIANA DI POLO [1,2]

1: Centre de recherche du Centre Hospitalier de l'Université de Montreal (CRCHUM)
2: Department of Neuroscience, Faculty of medicine, University of Montreal
3: Department of Pharmacology, Faculty of medicine, University of Montreal
4: Department of Medicine, Faculty of medicine, University of Montreal

Glaucoma is the main cause of irreversible blindness in the world. It is a neurodegenerative disease caused by the death of the retinal ganglion cells (RGCs), long projecting neurons that convey visual information from the retina to the brain. We previously showed that insulin daily eye drops administration to glaucomatous mice promoted RGC dendrite and synapse regeneration, restored RGC function, and improved vision. However, the modality of insulin application as eye drops can be limiting for insulin to reach the retina in human because of different eye anatomy. For this we transplanted Langerhans islets (LI), containing the specialized insulin-secreting beta-cells, in the eye to produce insulin locally. LI were isolated from mice pancreas using enzymatic digestion with collagenase. Around 50 islets were transplanted per eye using a 27G needle. The islets were transplanted directly on the iris without touching the lens nor blocking the iridocorneal angle. One month after transplantation, iris was imaged and insulin level in the anterior and posterior chamber was majored using radioimmunology. Insulin expression was significantly increased in the posterior and anterior chambers in transplanted eyes compared to sham operated eyes. Iris imaging using micron IV showed that Langerhans islets were stable and vascularised from the iris. Our finding suggests a potential role of LI transplantation in the eye to deliver insulin directly to the retina to promote neuronal regeneration during glaucoma. We will test whether LI transplantation improves RGC function and vision during glaucoma. Other potential molecules can be secreted by cells of LI to promote retinal neuron regeneration during glaucoma. We will explore further this possibility.

Metabolic Consequences of Exercise Cessation

LIAM G. HALL [1] , EMILIE KAYE [1] , GEORGE P. BROWNRIGG [1] , XIAOKE HU [1] , JOHN P. THYFAULT [2] , JAMES D. JOHNSON [1]

[1] Life Sciences Institute, Department of Cellular and Physiological Sciences, The University of British Columbia, Vancouver, BC

[2] KU Diabetes Institute and Departments of Cell Biology and Physiology and Internal Medicine-Endocrinology and Metabolism, University of Kansas Medical Center, Kansas City, KS

Transitioning from regular physical activity to inactivity increases risk factors for type 2 diabetes such as insulin resistance, hyperinsulinemia, and obesity, however, the underlying mechanisms remain poorly understood.

To investigate metabolic responses following a transition to inactivity, female and male C57BL/6J mice were fed a high fat/high sucrose diet from 12 weeks of age and housed under thermoneutral conditions (28°C). Mice had access to a running wheel for 4 weeks, prior to the wheel being locked for 7 days. Active and sedentary control groups were also included.

Exercise cessation for 7 days led to a rapid increase in adiposity, which approached levels seen in sedentary mice, and an elevation in fasting insulin to sedentary levels. Exercise cessation in male mice led to higher fasting glucose levels in comparison to both sedentary and active control groups, while glucose tolerance in female mice was comparable to that of sedentary mice after only 7 days of exercise cessation. Beta-cell mass was highest in mice following 7 days of wheel lock while beta-cell proliferation was found to be lower in active mice but increase to sedentary levels following exercise cessation.

Exercise cessation can rapidly increase risk factors for diabetes such as hyperinsulinemia, impaired glucose tolerance and increased adiposity. Current studies are now using this model to study the causal role of hyperinsulinemia in the development of metabolic dysfunction and the underlying mechanisms by which exercise and exercise cessation modify islet function and alter the risk of diabetes.

Functional characterization of TMEM55A on α/β cell exocytosis

Xiong Liu, Aliya F. Spigelman, Nancy Smith, Kunimasa Suzuki, Patrick E. MacDonald

Department of Pharmacology, University of Alberta, Edmonton, AB T6G 2E1, Canada
Alberta Diabetes Institute, University of Alberta, Edmonton, AB T6G 2E1, Canada

Background: Diabetes is associated with the dysfunction of glucagon-producing pancreatic islet α cells and insulin-producing β cells. We previously found that the expression of *TMEM55A*, a lipid phosphatase which converts phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol 5 Phosphate (PI5P), is positively correlated with α cell glucagon exocytosis, while negatively correlated with β cell insulin exocytosis through the patch-seq technique. Whether TMEM55A regulates exocytosis in α/β cells remains unknown.
Hypothesis: We hypothesize that TMEM55A controls α/β cell exocytosis by regulating PIP2/PIP5 signaling.
Results: We found that α cells transfected with si-*TMEM55A* show decreased exocytosis, consistent with the direction of correlation from patch-seq, without affecting calcium currents, compared with scrambled siRNA (n = 29, 31 cells, from 5 donors). Consistent with this, we also found that increasing PIP2, the substrate of TMEM55A, decreases α cell exocytosis (n = 23, 20 cells, from 4 donors), and that PI5P, the product of TMEM55A, highly increases α cell exocytosis (n = 8, 10 cells, from 2 donors). Consistently, PIP2 and PI5P treated islets demonstrated decreased

and increased glucagon secretion, respectively. Interestingly, *TMEM55A* knockdown had no significant effect on β cell exocytosis (n = 28, 25 cells, from 5 donors).
Conclusion: TMEM55A positively regulates α cell exocytosis, while seems to have no obvious effect on β cell exocytosis. The mechanism by which TMEM55A regulates α cell exocytosis through changing PIP2 or PI5P levels remains to be identified.

Session 2: The Metabolic Life and Death of the Pancreatic Beta Cell

Glycine Receptor Activity in β Cells Is Downregulated in Type 2 Diabetes and After High Glucose Culture

AMANDA SCHUKARUCHA GOMES, KUNIMASA SUZUKI , ALIYA F. SPIGELMAN , PATRICK E. MACDONALD

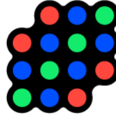
Alberta Diabetes Institute, Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada.

Background: Glycine Receptors (GlyRs) are present in human β cells, but their activity is impaired in type 2 diabetes (T2D) by an unknown mechanism. We investigated if the GlyR dysfunction in T2D is caused by hyperglycemia.
Methods: Islets from donors with or without T2D were used to quantify GlyR subunit mRNA splice variants and to measure GlyR-mediated currents from β cells using whole-cell patch-clamp. Current was also recorded after culture in 5.5 or 15 mmol/L glucose for 2 days. Insulin secretion from human islets was measured with and without 10 µmol/L strychnine, a GlyR antagonist.
Results: The glycine-evoked currents in β cells from donors with T2D (-0.81±0.51 pA/pF, n=7) were smaller than those from donors without diabetes (-12.72±2.38 pA/pF, n=29; p<0.001). The β cells cultured in 15 mmol/L glucose for 2 days had decreased glycine-evoked currents (-9.18±1.86 pA/pF, n=18, control: -17.63±3.53 pA/pF, n=14; p<0.05). The expression of most GlyR subunit mRNA splice variants was decreased in islets of donors with T2D. Glucose-stimulated insulin secretion (AUC=23.83±2.43) was reduced when 10 µmol/L strychnine was present (AUC=13.58±1.10;p<0.05).
Conclusions: Glycine-evoked currents in β cells are decreased after 2 days of culture with high glucose, showing that hyperglycemia is capable of modulating GlyRs. They are also decreased in T2D, where we find a decrease in overall GlyR gene expression, but not a shift in GlyR mRNA splicing. In addition, inhibiting the GlyR reduces glucose-stimulated insulin secretion.

Role of Ferroptosis and Mitochondrial Turnover in Glucocorticoids-Induced ß Cell Failure

ELISA MOLSTAD [1,2], SHERILYNE KETSIA MVOLO [1,2], ZENAB GILL [2,3], LEAH ISAAC [2], MERIEM BAADI [1,2], ANNE RINNA KOUASSI-DJAN [1,2], MOURAD FERDAOUSSI [1,2]

[1]Faculté Saint-Jean ; [2]Alberta Diabetes Institute, University of Alberta. [3]McMaster University



Introduction: Glucocorticoids are commonly used for their anti-inflammatory properties, but their chronic administration can lead to diabetes development. In non-ß cells, glucocorticoids trigger ferroptosis, a programmed cell death mechanism. Our research aims to explore the role of ferroptosis and its downstream mechanism in mediating glucocorticoid-induced ß cell dysfunction and death.

Methods and Results: The impairment of glucose-induced insulin secretion, as measured by ELISA in mouse islets treated with glucocorticoids dexamethasone (200 nM) and prednisone (1 µM), was restored by the ferroptosis inhibitor Liproxstatin (5 µM). Our preliminary data showed that the exposure of the insulin-secreting cell line INS832/13 to dexamethasone or prednisone, as well as to ferroptosis activator RSL3 (1 µM), reduced the expression of glutathione peroxidase 4 (GPx4) protein levels. GPx4 is a hallmark of ferroptosis that uses glutathione to reduce the oxidized form of lipids. Additionally, ferroptosis activator impaired mitochondrial fission and fusion protein markers in ß cells.

Discussion: Our data suggests that the ferroptosis pathway is, at least in part, mediating glucocorticoids-induced ß cell failure. We also propose that ferroptosis may mediate these effects by reducing mitochondrial fusion and fission turnover mechanisms. Together, these findings provide new perspectives for investigating the underlying mechanisms behind the diabetogenic effects of glucocorticoids.

The Role of Endoplasmic Reticulum Gla Protein (ERGP) in Beta-Cell Function and Cellular Calcium Regulation

KEVIN GUO [1,2], JULIE LACOMBE [1], LAURA QUIRION [3,4], JEAN-FRANÇOIS CÔTÉ [3,4], and MATHIEU FERRON [1,2,4]

[1]Molecular Physiology Research Unit, Institut de Recherches Cliniques de Montréal; [2]Division of Experimental Medicine, McGill University; [3]Cytoskeletal Organization and Cell Migration Research Unit, Institut de Recherches Cliniques de Montréal; [4]Programmes de biologie moléculaire, Université de Montréal

Vitamin K (VK) is an essential cofactor for gamma-carboxylase (GGCX), which converts glutamic acid (Glu) into gamma-carboxyglutamic acid (Gla) residues in proteins within the endoplasmic reticulum (ER). Clinical studies have implicated VK in the pathophysiology of diabetes, and our data indicate that beta-cells lacking GGCX fail to adapt their insulin secretion in response to glucose in the context of age- or diet-induced beta-cell stress. We identified ER Gla protein (ERGP) as a gamma-carboxylated protein regulating store-operated calcium entry that prevent calcium overfilling and beta-cell dysfunction. Our current objectives are: 1) to decipher the molecular mechanism by which gamma-carboxylated ERGP prevents cellular calcium overfilling by identifying its protein interactors; and 2) to determine the effect of the loss-of-function of ERGP in beta-cell function and calcium dynamics.

To identify ERGP interactors, we are using BioID, a protein-proximity assay. In this assay, the interactors are labeled via biotinylation by the miniTurbo protein fused to ERGP and identified by proteomics. We have generated mice with a specific deletion of ERGP in beta-cells ($Ergp^{f/f}$; *Ins1-Cre)* and insulin secretion in response to glucose will be analyzed in these animals. Calcium

dynamics in isolated *Ergp$^{f/f}$; Ins1-Cre* islets will be measured by microscopy. Preliminary data using *Ggcx$^{f/f}$; Ins1-Cre* islets suggest that gamma-carboxylated ERGP is necessary to maintain frequency and amplitude of calcium oscillations.

These experiments will shed light on the mechanisms by which ERGP maintains beta-cell function and calcium dynamics.

Cisplatin impairs β-cell health and function in mice

LAHARI BASU[1], MA. ENRICA ANGELA CHING[2], JANA PALANIYANDI[1], MYRIAM P. HOYECK[1], ANTONIO A. HANSON[1], KAYLEIGH R. C. RICK[1] , JAN A. MENNIGEN[2], JENNIFER E. BRUIN[1]

[1]Department of Biology; Institute of Biochemistry, Carleton University, Ottawa, ON, Canada; [2]Department of Biology, University of Ottawa, Ottawa, ON, Canada

Cancer survivors have an increased risk of developing Type 2 diabetes compared to the general population. Our research aims to determine if, and how, cisplatin, a commonly used chemotherapeutic agent, causes off-target damage to pancreatic β-cells, thus contributing to diabetes risk in cancer survivors. We first treated primary isolated mouse islets with 10 μM cisplatin or a vehicle control for 48-hours *in vitro*. Cisplatin-exposed islets displayed dysregulated insulin secretion, decreased oxygen consumption, and reduced expression of insulin and proprotein convertase genes. In a preliminary *in vivo* study, histological analyses showed that 2 weeks of cisplatin exposure (2 mg/kg/d every other day) in chow-fed male mice increased expression of DNA damage markers and accumulation of cytoplasmic proinsulin immunoreactivity in β-cells compared to vehicle-exposed controls. Next, male and female mice were fed either a standard chow or 45% high-fat diet (HFD) and exposed to the same vehicle or cisplatin treatment regimen as before. At 1-week post-treatment, male cisplatin-exposed chow-fed mice were hyperglycemic and hypoinsulinemic compared to vehicle-exposed chow-fed controls. In HFD-fed males, cisplatin-exposed mice were also hypoinsulinemic, but normoglycemic and more insulin sensitive than vehicle-exposed mice. Cisplatin exposure did not affect glucose tolerance, insulin sensitivity, or plasma insulin levels in female chow-fed mice; however, cisplatin caused hypoinsulinemia in HFD-fed females relative to vehicle-exposed controls. We are in the process of longer-term tracking for this mouse study, but our data thus far suggest that cisplatin treatment acutely disrupts rodent β-cell function.

Knockout of NCK1 in pancreatic islets lower insulin secretion in female mice.

LAURE MONTEILLET[1,2], NATHALIE JOUVET[1] , CINDY BALDWIN[1], JENNIFER L. ESTALL[1,2]

[1]Institut de Recherches Cliniques de Montréal, Montréal, Quebec, Canada ; [2]Division of Experimental Medicine, Department of Medicine, McGill University, Montréal, Quebec, Canada.

Preventing pancreatic β-cell failure could help treat diabetes. Silencing of *Nck1* in β-cell lines by enhancing the UPR pathway, improves β-cell function. Therefore, we hypothesized that knockout of *Nck1* in mouse islets would prevent β-cell failure.
We generated mice with a β-cell specific knock-out of *Nck1* (NCK1-βKO mice). Insulin secretion was assessed in KO mice and Cre controls, fed a chow or high fat/high sucrose (HFHS) diet. UPR pathway was analyzed in primary islets.
Under chow diet, female NCK1-βKO mice had lower insulin secretion and improved insulin sensitivity compared to control mice resulting in better glucose tolerance. Insulin content, insulin gene expression and UPR pathway in primary islets were similar. However, male NCK1-βKO mice had the same response to glucose (glycemia and insulin secretion) and insulin sensitivity compared to control mice despite a decrease in insulin gene expression and UPR pathway in primary islets.
Under HFHS diet female NCK1-βKO mice also exhibited lower insulin secretion and improved insulin sensitivity compared to control mice but had impaired glucose tolerance. This was associated with lower insulin gene expression and UPR pathway in primary islets. In contrast male NCK1-βKO mice exhibited similar insulin secretion in response to glucose as control mice, without changes in glucose tolerance and UPR pathway in primary islets.
Thus, in female mice, silencing *Nck1* in pancreatic β-cells decreases insulin secretion, which could reduce β-cell burden under basal conditions but impair β-cell adaptability to metabolic stress, promoting diabetes.

Exercise decreases senescence markers in mouse and human pancreatic islets through AMPK

Priscila Carapeto, Jiho Kahng, Ana Alves-Wagner, Roeland Middelbeek, Michael Hirshman, Guy A. Rutter, Laurie Goodyear, Cristina Aguayo-Mazzucato.

Increased β-cell senescence contributes to the development of type 2 diabetes (T2D). Exercise is critical in the treatment of T2D and can attenuate aging-associated cellular changes, but its effects on β-cell senescence are unknown. Using two mouse models of insulin resistance, we showed that exercise prevented and reversed β-cell senescence. Mechanistic studies revealed that these effects were mediated by exercise-induced increases in serum glucagon leading to AMPK activation in ⍰-cells. Nuclear translocation of NRF2 in mouse islets after exercise and its inversely proportional regulation of p16Ink4a, suggested its role as a molecular mediator between AMPK activation and cellular senescence. Treatment of human donor islets with serum obtained pre- and post- 10-week exercise training in people with and without T2D, showed that these findings can be translated into humans. In conclusion, exercise decreased senescence in pancreatic islets through a novel pathway, involving changes in glucagon and AMPK, with important therapeutic implications for T2DM.

The Impact of the Circulating Biomarker Nogo receptor (NogoR) on Pancreatic Beta Cell Survival

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Type 2 diabetes affects almost 10 % of westernised populations. Using a multi-omics approach in multiple cohorts of patients with type 2 diabetes within the EU-supported Rhapsody consortium, our lab has previously identified circulating biomarkers associated with disease progression.

One of the proteins identified was soluble NogoR. Encoded by the RTN4R gene, NogoR is predominantly expressed in the central nervous system where it is involved in inhibiting axonal outgrowth through interaction with its ligands. These include the myelin-derived proteins Nogo-A, -B and -C, which are encoded by RTN4. Our previous results showed that NogoR incubation induced apoptosis of mice and human pancreatic islets. Hence, we will determine the cellular mechanisms through which NogoR impact pancreatic beta cell survival. Our current hypothesis is that the apoptotic effects of NogoR are mediated by competing with the binding to its ligands. By western blot, we found that while NogoA shows more variable levels between mice and human pancreatic islets, NogoB is expressed in both. Consequently, we propose that NogoB might mediate the apoptotic effects of NogoR. These findings might help in the discovery of a new apoptotic pathway in pancreatic islets that can be targeted therapeutically.

Mechanisms of Control of Pancreatic β-cell Proliferation by Nutrients

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Increasing β-cell mass is a promising therapeutic approach to compensate for the loss of β cells in diabetes. Under nutrient excess, β-cell mass expands to maintain normoglycemia, partly via proliferation. Recently we showed 1- that the mono-unsaturated fatty acid oleate increases β-cell proliferation under high glucose, and 2- that reactive oxygen species (ROS), the antioxidant enzymes peroxiredoxins, and the transcription factor MYC are involved in oleate-induced β-cell proliferation.
This project hypothesizes that ROS/peroxiredoxin signaling via MAPK/MYC promotes oleate-induced β-cell proliferation.
Isolated adult male rat islets were exposed to glucose with or without oleate for 48 hours. Proliferative β cells were detected by staining for c-peptide and the proliferative marker MKi67 and quantified by flow cytometry.
To assess the effect of glucose concentration on oleate-induced β-cell proliferation in rat islets, a glucose-dose response with or without oleate was performed. Oleate (0.5mmol/L) dose-dependently potentiated β-cell proliferation at all glucose concentrations tested (2.8 mmol/L: 3.9±0.6%; 11.1 mmol/L: 2.7±0.5; 16.7 mmol/L: 2.7±0.3%; n=4). The MAPK inhibitor U0126 (0.01 mmol/L) decreased oleate-induced β-cell proliferation from 8.2±1.8% to 2.1±0.6% (n=6).

We conclude that oleate increases β-cell proliferation independently of the glucose concentration, and that the MAPK signaling pathway is likely a downstream effector of ROS/peroxiredoxin signaling in oleate-induced β-cell proliferation.

Insulin secretion on-a-chip (InsC-chip) to measure the dynamics of secretion and metabolism from individual islets

Yufeng Wang, Romario Regeenes, Mahnoor Memon, Nitya Gulati, Jonathan V. Rocheleau

First-phase glucose-stimulated insulin secretion is mechanistically linked to type 2 diabetes yet the underlying metabolism is difficult to discern due to the transient nature of the response and significant islet-to-islet variability. Conventional perifusion systems measure secretion pooled from many islets. On the other hand, currently available islet-on-a-chips either pool islet secretion and/or require off-chip measurement (e.g. ELISA), both of which blur the insulin response. Here, we miniaturized a fluorescence anisotropy immunoassay onto a microfluidic device to measure mouse C-peptide secretion from individual islets as a surrogate for insulin (InsC-chip). This method measures secretion from up to four islets at a time with ~7 s resolution while providing an optical window for real-time live cell imaging. Using the InsC-chip, we reveal for the first time two glucose-dependent peaks of insulin secretion (i.e., a double peak) within the classically defined first phase (<10 min). By combining real-time secretion and live cell imaging, we show islets transition from glycolytic to OxPhos-driven metabolism at the nadir of the peaks. Overall, these data validate the InsC-chip to measure glucose-stimulated insulin secretion with outstanding resolution while revealing new dynamics in secretion defined by a shift in glucose metabolism. We envision this microfluidic platform will allow on-chip measurement of different islet secretion. Next, we aim to design a human C-peptide sensor to investigate the dynamics of secretion from human islets, and a glucagon sensor to simultaneously probe glucose-stimulated glucagon secretion.

Session 3: Novel Approaches for T1D Treatment

Examining Notch Signalling To Improve Human Stem Cell-Derived Pancreatic Cell Yield

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One challenge presented with islet-like cell production from pluripotent stem cells (PSCs) is the low final number of beta-like cells. Identifying signalling pathways that promote endocrine differentiation from PSCs in vitro could be used to produce a reliable cellular therapy for people living with type 1 diabetes. One candidate pathway is through Notch signalling, which affects early pancreatic cell expansion and endocrine commitment in vivo. Transcripts for Notch receptors and ligands are up-regulated during PSC-derived early pancreatic commitment, suggesting that endogenous signalling may occur at these stages. To examine whether further activating Notch promotes pancreatic cell expansion, we utilized OP9 stromal cells expressing Notch ligands delta-

like ligand 1 (DLL1) or DLL4. Control and DLL1/4 OP9 cells were co- cultured with PDX1 + cells and assessed by flow cytometric and RT-qPCR analysis for pancreatic and proliferative markers to determine differentiation and expansion. PDX1 + cells cultured on all OP9 conditions were able to expand following passage and maintained PDX1 + pancreatic cell fate, but no differences in cell proliferation were found between treatments. PDX1 + / OP9 co-cultures were able to differentiate to beta-like cells and produced 2-3 times more total cells than control differentiations. Co-culture using PDX1 + /NKX6.1 + cells, which represent a later stage of our differentiation protocol, promoted expansion on OP9 cells but reduced PDX1 + /NKX6.1 + cell commitment. Experiments using a stromal cell-free system to induce Notch signalling are in progress and will measure stromal-free Notch effects on pancreatic cell expansion and differentiation.

Generating human stem cell-derived β-cells that attract T regulatory cells

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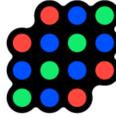
Type 1 diabetes is an autoimmune disease where functional insulin-producing β-cells are destroyed, leading to hyperglycemia. Although islet transplantation has potential to become an alternative therapy to lifelong insulin injections, there are several challenges to overcome. At present, patients who receive islet transplants require immunosuppressants to prevent immune destruction of the transplanted cells, causing risk for infection. One approach to attenuate allograft rejection is by attracting immunosuppressive T regulatory cells (Tregs) to the transplant by expressing chemokines. We aim to determine if transplanted stem cell-derived β-cells (sc-β-cells) genetically engineered to secrete the chemokine CCL22 will cause Tregs to migrate to the site of the graft. We have generated embryonic stem cells with CCL22 inserted under the insulin promoter, and using a 7-stage protocol, differentiated them into insulin-producing sc-β-cells. We verified that the insertion of CCL22 does not affect the differentiation or functionality of the sc- β-cells. The bioactivity of CCL22 from sc-β-cells was assessed in vitro with a CCL22 immunoassay and Treg migration assay. The CCL22+ sc-β-cells were transplanted into either the anterior chamber of the eye or kidney capsule of immunodeficient, diabetic mice infused with human Tregs. Our group has shown that the engineered sc-β-cells are able to improve blood glucose levels and there is circulating c-peptide. We have confirmed Treg homing to the site by both bioluminescent imaging and immunostaining. In our model, we show that more Tregs are attracted to grafts expressing CCL22 than controls.



Investigating the Role of Angiomotin in HPSC-Directed Pancreatic Endocrine Differentiation

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Current differentiation protocols generating human pluripotent stem cell (hPSC)-derived islets as a renewable cell replacement therapy for type 1 diabetes remain to be optimized. Recently, our lab showed that pancreatic endocrine differentiation is enhanced with tankyrase inhibition. Upregulation of the scaffold protein angiomotin (AMOT) is correlated with this finding and interestingly, AMOT participates in other pathways reported to influence endocrine commitment including actin cytoskeleton, Hippo-YAP, and mechanotransduction. How AMOT directly plays a role in endocrine differentiation is unknown. Here, I characterized AMOT expression levels during endocrine differentiation by western blot and RT-QPCR analysis. To monitor endocrine commitment, I performed a kinetic analysis of Neurogenin3, NKX2-2, and Chromogranin A using flow cytometry. As expected, endocrine commitment is accompanied by upregulation of all three endocrine precursor markers. This can be enhanced by continuous tankyrase inhibition which remarkably, is also associated with higher AMOT expression, specifically the YAP-interacting p130 isoform. Current and future studies will focus on the hypothesis that increased AMOT may promote endocrine commitment. To test this, I will use lipid nanoparticles (LNP) to deliver *AMOT* mRNA into pancreatic progenitors to see whether AMOT overexpression can further enhance endocrinogenesis. Preliminary data revealed that LNPs can be used to efficiently deliver *GFP* mRNA into pancreatic progenitors (>90% GFP^{+} cells post-transfection), demonstrating the feasibility of transfecting *AMOT* mRNA. These studies will ultimately provide novel insights to efficiently generate islets while recognizing AMOT as a link to several pathways involved in fetal pancreatic development.

Transplantation of pramlintide-expressing stem cell-derived beta cells to mitigate amyloid associated islet transplant failure

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Transplanting insulin-producing human embryonic stem cell-derived β cells (SC-β) is a promising alternative to islet transplantation for people with type 1 diabetes (T1D). With advances in differentiation protocols and clinical trials, SC-β cells may obviate the need for organ donors, although the long-term survival and function of these cells following transplantation still needs

further investigation. Aggregation of islet amyloid polypeptide (IAPP), a hormone co-secreted with insulin from β cells, and the major component of cytotoxic islet amyloid in type 2 diabetes, has been implicated in islet transplant failure. Pramlintide is a non-aggregating, non-toxic human IAPP (hIAPP) analogue that is used clinically as an adjunct therapy in T1D. Using CRISPR-Cas9, we modified the hIAPP sequence in H1 SCs to express pramlintide instead of endogenous hIAPP. Pramlintide, along with wild-type hIAPP-expressing SCs (WT), were differentiated into mature, insulin-producing SC-β cells. Transcript analysis indicated both WT and pramlintide-expressing SC-β cells expressed markers of β-cell maturation and function identical to immature human islets. Glucose-stimulated insulin secretion suggested maturation with long-term culture, and a $hIAPP_{1-37}$ ELISA indicated pramlintide SC-β cells secrete an altered, non-amyloidogenic IAPP form. Diabetic immunodeficient mice transplanted with WT or pramlintide SC-β cells showed a higher C-peptide response in mice with pramlintide SC-β cells at 6-weeks with a fast-refeed test. Our preliminary data suggest pramlintide expression does not adversely impact maturation of SC-β cells and that these cells may show higher C-peptide production post-transplant. Future studies will evaluate the impact of encapsulated SC-β cell transplants in immune-deficient and immune-competent diabetic mice.

A Novel Small-Molecule Activator of Lyn Kinase for The Treatment of Type 1 Diabetes

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Type 1 diabetes (T1D) is caused by autoimmune-meditated destruction of pancreatic β-cells. It has been shown that individuals with long-standing T1D still have surviving functional β-cells. This provides opportunities to restore functional β-cell mass to delay or reverse T1D. Our recent proof of-concept study has shown that MLR-1023, an activator of Lyn tyrosine kinase, stimulates β-cell mass expansion in T1D mouse models and restores normoglycemia. We herein sought to test the hypothesis that MLR-1023 could reduce islet autoimmunity, and that this action contributes to its anti-diabetes properties. Surprisingly, a short treatment of 7 days with MLR1023 was sufficient to reduce islet insulitis scores in diabetic NOD mice. MLR1023-treated animals had more remaining islets in the pancreas. They also displayed a significant reduction in the number of $B220^+$ B cells, $CD4^+$ T cells, $CD8^+$ T cells, and $CD68^+$ macrophage surrounding or infiltrating islets. These results suggest an anti-inflammatory effect of MLR1023. The number of $FoxP3^+$ Treg was not significantly altered between the two groups, whether it be in the islets or pancreatic lymph nodes. Our preliminary data in isolated human islets showed that MLR1023 attenuates cytokine-induced stress markers and reduces IL-1β expression. Altogether, our results indicate that MLR1023 simultaneously promotes β-cell mass expansion and reduces islet inflammation, thereby correcting two defects that underlie T1D. This greatly increases our interest in MLR1023 as a potential anti-T1D medication.

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

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For the past century, insulin was the sole Federal Drug Administration (FDA) approved type 1 diabetes (T1D) treatment. In 2022, Teplizumab, an anti-CD3 immunotherapy, received approval to delay symptomatic T1D, although its exact mechanisms remain unclear. Teplizumab improves beta cell function, suggesting an impact on beta cell stress. We hypothesize Teplizumab may reduce beta cell senescence, a stress pathway which may adversely affect beta cell function.
Female non-obese diabetic (NOD) mice received weekly doses of anti-CD3 or control treatment from weeks 9-13. Intraperitoneal glucose tolerance tests were conducted to assess beta cell function. Senescent beta cell frequency and gene/protein expression were evaluated using immunohistochemistry (IHC), reverse transcriptase quantitative polymerase chain reaction (RT-qPCR), and Luminex, respectively. Treatments were validated using flow cytometry.
Significant modulation of total CD3+ T cells ($p=0.0020$) and changes in antigen-specific T cells occurred in the anti-CD3 mice. IHC revealed increased insulin+ stained area ($p=0.0104$), reduced insulitis and fewer senescent beta cells ($p=0<0.0005$) in the anti-CD3 mice but no difference in markers of beta cell identity or unfolded protein response.
Our findings in NOD mice suggest a novel mechanism of anti-CD3 action during late pre-diabetic stages. Anti-CD3's modulation of cytotoxic T cells may slow disease progression by limiting senescent beta cell accumulation. These findings raise questions about the interplay between cytotoxic T cell depletion, beta cell function, and senescence in T1D while offering exciting avenues to investigate these multifaceted relationships.

Development of a BRET-Based High-Throughput Screening Approach to Discover Anti-Type I Diabetes Compounds from Previously-Approved Drugs

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Background and aim: In type I diabetes (T1D), autoreactive CD8+ T cells are the main cell type responsible for causing beta-cell death; hence, the depletion of autoreactive T cells could protect beta-cells. Our lab has demonstrated that interactions between scaffold protein 14-3-3(z)eta and BCL-2 family proteins, including BAD, prevent cell apoptosis. Thus, we hypothesize that disrupting 14-3-3z:BAD interactions in autoreactive T cells could induce apoptosis and might serve as a novel drug target for treating T1D.

Material and methods: We developed a living-cell bioluminescence resonance energy transfer (BRET) sensor to screen for compounds that disrupt 14-3-3z:BAD interactions from an FDA-approved drug library in a high-throughput format. The identified compounds were assessed for their ability to induce cell death and apoptosis using a high-content imaging system.
Results: Knocking out 14-3-3z in CD8 T cells of female mice had protective effects against multiple low-dose streptozotocin-induced diabetes. Inhibition of 14-3-3 promoted the translocation of BAD from the cytoplasm to mitochondria. We identified 47 out of 1,971 drugs, and these compounds will be tested in primary CD8+ T cells.
Conclusion: We have successfully developed a novel BRET sensor that can detect 14-3-3z:BAD interactions, enabling screening for pro-apoptotic compounds in living cells. The results of our screening may lead to the discovery and repurposing of drugs that delay the progression of T1D by killing cytotoxic T cells.

Utilizing AAV8-Ins1-Cre Mediated Gene Deletion to Investigate Prohormone Processing Deficiencies in Non-Obese Diabetic Mice

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Pancreatic beta cells produce the prohormone, proinsulin, which is processed to its mature biologically active form, insulin, by the prohormone convertases PC1/3 and PC2, and carboxypeptidase E (CPE). Individuals living with type 1 diabetes (T1D) exhibit persistent secretion of proinsulin, indicating that processing of beta-cell prohormones is impaired in T1D. Indeed, non-obese diabetic (NOD) mice, a model of autoimmune diabetes, display elevated levels of proinsulin early in life. However, the contribution of processing enzyme loss to diabetes onset remains unknown. We hypothesize that knockout of the processing enzymes Pc1/3 and Cpe will exacerbate impaired prohormone processing in NOD mice, and lead to earlier and/or increased diabetes incidence. NOD mice with floxed genes, *Pcsk1* and *Cpe,* are being generated. To induce beta-cell specific knockout of *Pcsk1* and *Cpe in vivo*, an adeno-associated virus expressing Cre recombinase under the control of the insulin 1 promoter, AAV8-Ins1-Cre, will be introduced surgically into the pancreatic ducts of prediabetic female and male NOD mice. Diabetes progression will be measured by recording blood glucose levels and body weight. Proinsulin and C-peptide will be detected by ELISA. Preliminary data demonstrate efficient beta-cell recombination at low ($1x10^{11}$ vgp/mouse) and high ($5x10^{11}$ vgp/mouse) doses of AAV8-Ins1-Cre administered intra-ductally (88.7% and 93.8%, respectively) and intraperitoneally ($1x10^{12}$ vgp/mouse; 93.8%). This study will enable better

understanding of the contribution of loss of prohormone processing enzymes to the pathogenesis of T1D.

Session 4A: Polymorphisms, MODYs and T2D

Characterization of a Common CERS2 Polymorphism and Links to type 2 diabetes Development

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Ceramide Synthase 2 (CERS2) gene encodes for a dihydroceramide synthase, which is involved in the biosynthesis process of very-long-chain sphingolipids from ceramide, and the downregulation of such sphingolipid metabolism pathway is closely associated with the early-stage type 2 diabetes (T2D) pathogenesis. A common single nucleotide polymorphism (SNP; rs267738) within such gene was found strongly associated with reduced CERS2 enzyme functionalities using humanized knock-in *Cers2* polymorphism mice model, implying the potential metabolic dysfunction, and increased risk for T2D development. In this study, we conducted both intensive in vivo and ex vivo studies to examine the glycemic impact of the female humanized *Cers2* polymorphism mice on a C57BL/6 background at 10-14 weeks on a normal chow diet, and wildtype C57BL/6 mice were used as controls. No significant difference was found in the insulin tolerance tests, but *Cers2* polymorphism displayed mild glucose intolerance during glucose tolerance tests (p=0.0073). Interestingly, ex vivo studies revealed greatly reduced insulin-secretion capacity in *Cers2* polymorphism by performing glucose-stimulated insulin secretion assay (p=0.0021), whereas no morphological difference of the isolated islets was found. These data suggest the effect of an SNP in a critical risk factor gene on alternating metabolic functions, especially among the female population. Altogether, this study provides us with a more detailed appreciation of the partial loss of CERS2 functionalities and its associated risk for T2D due to the reduced insulin-secreting ability.

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

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Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) 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, SNP rs8192678) 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). 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. 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. 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.

INVESTIGATING THE EFFECT OF THE HNF-1a G319S VARIANT ON ISLET FUNCTION AFTER PROLONGED FASTING

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

University of Manitoba, Physiology and Pathophysiology; Children's Hospital Research Institute of Manitoba; DREAM theme

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.

GK-mCardinal knock-in mice: a novel model of GK haploinsufficiency

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Glucokinase (GK) is a critical β cell glucose sensor, catalysing the flux-generating step of glycolysis, and heterogeneously present in rat and mouse islets. In order to image GK expression across the living islet, we have generated a mouse model in which the far, red-shifted fluorescent protein mCardinal is knocked-in at the endogenous GK locus. Our results show GK-mCardinal mice display abnormal glucose handling. GK-mCardinal represents a novel hypomorphic allele of the murine glucokinase gene. Both hetero- and homozygous carriers display a milder phenotype than null alleles [4,5,6] likely reflecting an aberrant splicing event with incomplete penetrance. This offers the prospect of monitoring islet function during the progression of disease in a model Mendelian diabetes, GK-MODY.

Session 4B: Beta Cell Communication and Islet Structure

Do highly-connected beta cell (i.e. "hubs") overlap with epigenetically-defined "BetaHi" cells?

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Background: Beta cell heterogeneity is important to ensure normal glucose-stimulated insulin release from the pancreatic islet. Recent work has described "Beta Hi" and "Beta Lo" populations defined by distinct epigenomic profiles and expression of the cell surface marker CD24. The extent to which these sub-groups may align with beta cell subpopulations from which calcium oscillations emanate ("leaders") or are coordinated ("hubs") is unclear.
Objective: To determine whether CD24+ cells ("Beta Hi"), overlaps with "hub" cells.
Methods : Islets were isolated from Ins-Cre GCaMP6 mice and then transfected with an adenovirus expressing Photo Activatable (PA)-mCherry. Calcium waves were recorded by confocal imaging and analysed in ImageJ and MATLAB. Highly-connected hubs were identified and labelled by

photoexcitation of PA-mCherry. Islets were then fixed, and an immunostaining was performed to identify CD24+ cells.

Results: In some, but not all, islets we were able to identify "hubs", representing approximately 10% of the beta cell population. CD24 immunoreactivity was correlated with insulin (Pearson's $r=0.68$ $p<0.05$; N= 5 islets from 2 mice), but was not enriched in previously-identified hubs.

Discussion: We were able to identify hubs and assess the levels of a cell marker in this subpopulation. Contrary to our expectations, CD24 was not enriched in hub cell populations, suggesting that hubs may form part of the "BetaLo", rather than a "BetaHi" population within the islet. Future studies will be needed to assess to which sub-population "leader" cells belong, and whether these assignations are altered in models of type 2 diabetes.

Investigating how Pancreatic Islet Architecture Impacts Function

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Islets of Langerhans are clusters of endocrine cells within the pancreas which detect nutrients in the blood and release hormones to regulate blood glucose levels and nutrient uptake. These islets contain five endocrine cell types that produce different hormones: alpha (glucagon), beta (insulin), delta (somatostatin), epsilon (ghrelin), and gamma (pancreatic polypeptide), with alpha and beta cells comprising ~90% of islet mass. Despite different species having remarkably similar islet sizes, the cytoarchitecture – the spatial organization of cell types – varies between species, within individuals, and between healthy and diabetic states. As these cell types communicate via physical and hormonal interactions and cytoarchitecture has been associated with changes in islet calcium dynamics, variations in cytoarchitecture are likely critical to islet function and glycemic regulation; yet we do not understand the origin of cytoarchitectural differences nor their effects. We are making pseudoislets from stem cell derived alpha and beta cells using cell lines with insulin and glucagon reporters and primary human islets infected with a viral vector containing insulin and glucagon reporters. We are studying the role of islet size, cell ratio, and membrane protein expression on cytoarchitecture and islet function. This knowledge will help understand why cytoarchitecture varies and may help design cell therapies with cytoarchitectures which improve glycemic control.

Differing stabilities of "Leader" and "Hub" β-cells within the pancreatic islet in vivo

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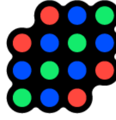

We and others have identified subpopulations of β-cells termed "leaders" and "hubs". Whereas the former are the first cells to show an increase in intracellular Ca^{2+} during islet Ca^{2+} waves, the latter are essential for the coordination of Ca^{2+} oscillations. Whether these groups simply reflect transient functional states, or are stable subpopulations, remains unclear. Here, we employed confocal calcium imaging (3-6 Hz), cell-tracking and longitudinal imaging *in vivo* to characterize β-cells stability and control the islet calcium dynamics. Adenoviral delivery of the photoactivable PA-mCherry allowed the identification and tracking of leader cells using "photopainting". *In vitro,* leader cells remained stable when imaged at 0 h and 24 h later (day 1) in 80% (4 mice). After islet engraftment into the anterior chamber of the eye (ACE), Ca^{2+} waves *in vivo* emanated from the same leader cells at days 0, 1, 7 and 14 days (*n*=6 islets imaged in 3 separate mice). In contrast, whereas 9.5% of cells were defined as hubs when examined *in vivo* on day 0, this fraction increased to 18.3% on day 1. The proportion of hubs/islets then decreased progressively at day 7 and day 14: 8.8% and 1.1% of the total respectively. These findings demonstrate that leader β-cells are a stable subpopulation *in vitro* and *in vivo,* consistent with the distinct transcriptomes and localization of these cells within the islet. The behavior of hubs is more complex, with the proportion and identity of these cells varying substantially *in vivo* over a two-week time frame.

Islet Paracrine Factors Direct Glucagon to Lysosomes in Pancreatic Alpha Cells

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Background: Glucagon secretion is inhibited by the paracrine factors, insulin, GABA and somatostatin. Our previous work has shown that glucagon secretion may be regulated through lysosomal trafficking by a neuronal protein, stathmin-2 (Stmn2). We hypothesize that insulin+GABA, and somatostatin, modulate glucagon secretion by increasing Stmn2-mediated lysosomal trafficking of glucagon in alpha cells.
Methods: AlphaTC1-6 cells were treated with 1 nM insulin+25 µM GABA, or 400 nM somatostatin for 24 h. Cells were fixed and stained for immunofluorescence for glucagon, Stmn2, the lysosomal marker LAMP1 or the granule exocytosis marker Syntaxin1A. Cells were divided into 3 regions of interest (ROIs): nuclear, intracellular and periphery. After image acquisition by confocal microscopy, the distribution of immunofluorescence intensities of co-localized pixels in ROIs from 12 cells per treatment group was determined by plot profiles in ImageJ Fiji. Data were analysed by two-way ANOVA and post hoc tests.
Results: Co-localization of glucagon and LAMP1, or glucagon and Syntaxin1A, redistributed significantly to the intracellular region with insulin and GABA ($p<0.0001$ for both treatments) and somatostatin ($p<0.0001$ for both). Colocalized Stmn2 and glucagon at the periphery was significantly decreased ($p<0.0001$) with insulin/GABA or somatostatin treatment. Transfection

with Stmn2-GFP significantly increased nuclear translocation of TFEB ($p<0.0001$), a transcriptional regulator of lysosomal biogenesis.

Conclusion: Our results demonstrate that paracrine factors may inhibit glucagon secretion through increased lysosomal trafficking, possibly mediated by Stmn2. Stmn2 may activate lysosomal biogenesis via TFEB, suggesting transcriptional mechanisms may be involved.

The long-chain fatty-acid receptor FFA4 stimulates insulin secretion via inhibition of somatostatin release from the delta cell

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The long-chain fatty-acid receptor FFA4 exerts beneficial effects on glucose homeostasis and insulin secretion and is considered a potential therapeutic target for type 2 diabetes. FFA4 is expressed in islets, but its precise mechanism of action remains unknown. Previous studies from our group suggest that FFA4 is expressed in delta cells and regulates somatostatin secretion.

The objective of this study was to test the hypothesis that FFA4 agonists indirectly stimulate insulin secretion via inhibition of somatostatin release. In 1-h static incubations of isolated, wild-type mouse islets, the FFA4 agonist CpdA dose-dependently potentiated glucose-induced insulin secretion from 2.2±0.2 to 3.4±0.2 % of insulin content at the concentration of 50 µM ($n=8$; $p<0.001$), and simultaneously decreased somatostatin secretion from 28.4±2.1 to 10.5±0.9 pM ($n=8$; $p<0.0001$). No effect of CpdA on insulin or somatostatin secretion was observed in islets from mice that do not express somatostatin (insulin: 3.2±0.1 vs 2.9±0.4, n=6, NS; somatostatin: 11.4±1.2 vs 11.9±1.2, n=6, NS). No sex differences were observed. Likewise, in delta-cell deficient islets isolated from diphtheria toxin-treated male mice expressing the diphtheria toxin receptor under the somatostatin promoter, no effect of CpdA was observed on insulin or somatostatin release (insulin: 4.9±0.9 vs 4.1±0.8, n=3, NS; somatostatin: 10.1±4.9 vs 9.2±5.3, n=6, NS).

We conclude that FFA4 stimulation of insulin secretion is exclusively mediated by inhibition of somatostatin secretion from delta cells.