CIRTN
Canadian Islet Research and Training Network

R2FIC
Réseau de recherche et formation sur les îlots du Canada

National Meeting
November 8-9, 2022

The Delta Hotel

Welcome! *From the CIRTN-R2FIC Leadership Group*

Welcome to the first ever Canadian Islet Research and Training Network – Reseau de Recherche et Formation sur les Ilots du Canada (CIRTN-R2FIC) National Meeting!

The past two years have seen the birth and growth of our network, and we are very excited for this opportunity to come together as a community in-person for the first time to connect, network, and share the exciting work that we are all doing. We are absolutely delighted to see so many established and budding islet biologists make the trek to Calgary from all over Canada. We have attendees from over 10 universities/research institutes and 5 provinces. Over the next two days, you can look forward to a fascinating keynote lecture from the esteemed Dr. Barbara Corkey (Boston), 40 outstanding trainee presentations, mentorship and skill-development workshops, fun networking opportunities, and exciting science across the spectrum of islet biology and diabetes research and care.

Importantly, an event like this is made possible by the contributions and hard work of many people and organizations. First and foremost we would like to thank our financial partners and sponsors (see pg. 7 for details) for making this meeting possible and allowing us to provide important incentives and perks to maximize our trainee involvement in this event.

We would also like to give special recognition to the CIRTN-R2FIC National Meeting Planning Committee (Pg. 5), who worked hard to plan the events of these two days, as well as integrate our event into the ULTRA Scientists Day (formerly the Diabetes Action Canada Trainee Day) and into the Diabetes Canada Professional Conference. The partnerships with these organizations/meetings deepen our learning experiences and expand the horizons of our network.

Finally, as an unofficial (and pandemic-delayed) kick-off for our network, we feel we should take this opportunity to also recognize that our network and this meeting wouldn't be possible without the hard work of the core CIRTN-R2FIC committees, including the Strategic Planning Committee (led by Dr. Vincent Poitout), the EDI Committee (led by Drs. Elizabeth Rideout and Gareth Lim), the Mentorship Committee (led by Dr. Mathieu Ferron) and the Trainee Volunteer Group (led by Noa Gang). Please see a full list of these committees in the pages below. We would also like to recognize Drs. Erin Mulvihill and Rob Screaton for their contributions to the development and implementation of our first-ever national graduate course in Islet Biology. In 2023 this course is going into its 3rd successful year and it has become a cornerstone of our training program.

We urge you to take a moment to check out the individuals involved in these committees, either in this program or on our website (islets.ca – thanks to Dr. Bruce Verchere for donating this awesome domain name!), and take the time to thank them over the next couple of days as you interact with them.

With all of that said, HAVE FUN, LEARN LOTS, and most importantly MAKE CONNECTIONS!

Sincerely,
The CIRTN-R2FIC Leadership Group:


Patrick MacDonald


Jenny Bruin


Jennifer Estall


Bruce Verchere


Jon Rocheleau


Christine Doucette

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

The Canadian Islet Research and Training Network (CIRTN) 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-R2FIC now includes over 50 laboratories across the country and more than 200 associated researchers and staff engaged in the study of the pancreatic islets of Langerhans.

Islets are micro-organs that produce insulin and other important endocrine hormones which help the body maintain normal blood sugar levels. Loss of islet cells, or impairment in their function, is a hallmark feature of diabetes. Notably, over 100 years ago insulin was discovered in Canada, changing the global landscape of diabetes research and treatment. 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 are committed to developing approaches to knowledge mobilization and engagement across Canada in a manner that fosters equity, diversity, and inclusivity.

LEADERSHIP GROUP

Patrick MacDonald (Chair; University of Alberta; ADI)
Jenny Bruin (Carleton University)
Christine Doucette (University of Manitoba, DREAM)
Jennifer Estall (Université de Montréal; IRCM)
Jonathan Rocheleau (University of Toronto; BBDC)
Bruce Verchere (University of British Columbia; BCCHR)
Rob Screaton (University of Toronto, Sunnybrook Research Institute; former member)

STRATEGIC PLANNING COMMITTEE

Vincent Poitout (Chair; Université de Montréal; CRCHUM)
Francis Lynn (University of British Columbia; BCCHR)
Erin Mulvihill (University of Ottawa)
Elizabeth Rideout (University of British Columbia)
Peter Thompson (University of Manitoba)
Noa Gang (Trainee Representative; Carleton University)

EQUITY, DIVERSITY & INCLUSION COMMITTEE

Gareth Lim (Co-Chair; Université de Montréal; CRCHUM)
Elizabeth Rideout (Co-Chair; University of British Columbia)
Tina Dafoe (University of Alberta)
Chris Peacocke (University of Alberta)
Cara Ellis (University of Alberta)
So-Yoo Won (University of Toronto)
Jasmine Maghera (University of Alberta)
Taylor Morriseau (University of Manitoba; former member)
Tamadher Alghamdi (former member)

MENTORSHIP COMMITTEE

Mathieu Ferron (Université de Montréal)
Jenny Bruin (Carleton University)
Andrew Pepper (University of Alberta)
Emily Hoffman (York University)
Nivedita Seshadri (University of Manitoba; former member)

TRAINEE VOLUNTEER GROUP

Noa Gang (Lead; Carleton University)
Hui Huang (University of Alberta)
So-Yoo Won (University of Toronto)
Emily Hoffman (York University)
Jana Palaniyandi (Carleton University)
Roozbeh Akbari Motlagh (University of Alberta)

Previous Trainee Volunteers:
Daemon Cline (University of British Columbia)
Nivedita Seshadri (University of Manitoba)
Tamadher Alghamdi

NATIONAL MEETING PLANNING COMMITEE

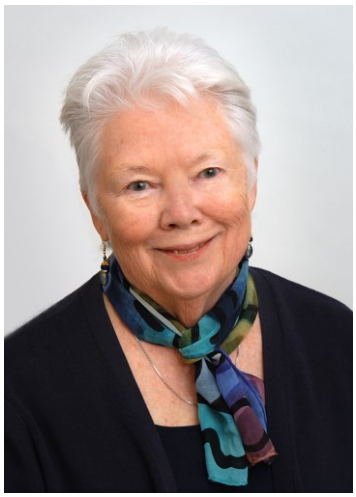
Christine Doucette (Chair; University of Manitoba; DREAM)
Chris Peacocke (CIRTN-R2FIC Administrative Coordinator)
Jean Buteau (University of Alberta; ADI)
Jennifer Estall (Université de Montréal; IRCM)
Carol Huang (University of Calgary)
Gareth Lim (Université de Montréal; CRCHUM)
Erin Mulvihill (University of Ottawa; Ottawa Heart Institute)
Cristina Nostro (University of Toronto; BBDC)
Andrew Pepper (University of Alberta; ADI)
Peter Light (University of Alberta; ADI)
Jonathan Rocheleau (University of Toronto; BBDC)
Mark Ungrin (University of Calgary)
Bruce Verchere (University of British Columbia; BCCHR)

NATIONAL ISLET COURSE DIRECTORS

Erin Mulvihill, (University of Ottawa; Ottawa Heart Institute)
Robert Screaton (Sunnybrook Research Institute; University of Toronto; BBDC)
Carrie Harber (University of Toronto, administrative support)
Chris Peacocke (University of Alberta, administrative support)

A bit about our Keynote Speaker…

Dr Barbara E. Corkey, PhD.



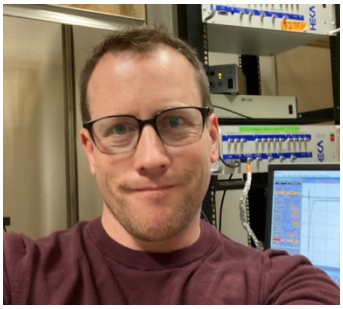
Dr. Barbara E. Corkey is Professor Emeritus of Medicine and Biochemistry at Boston University School of Medicine. Dr. Corkey has been a leader in the fields of islet biology, diabetes and obesity research for over 50 years. She was the Zoltan Kohn Professor of Medicine and Vice Chair for Research in the Department of Medicine at Boston University and has published ~200 related publications with 40 years of continuous government research support. Finally, Dr. Corkey has received numerous esteemed honors including the NIH MERIT Award, National Honorary Membership in Iota Sigma Pi, the National Honor Society of Women in Chemistry, Women in Science Lecturer at the Boston Museum of Science, the George Bray Founders Award of the Obesity Society, the Charles H. Best Lectureship and Award from the University of Toronto and the Banting Medal for Scientific Achievement from the American Diabetes Association.

Mentorship Awards…

Sponsored by the Alberta Diabetes Institute and the CRCHUM

CIRTN-R2FIC has decided to recognize two exceptional CIRTN-R2FIC members, one PI and one non-PI, at the National Meeting for their outstanding contributions to mentorship and training within the network over the past year. Each awardee 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.

The PI Mentorship Award goes to…. *Dr. Patrick MacDonald, University of Alberta*



Patrick completed his BSc at the University of Western Ontario in 1998 and his PhD at the University of Toronto under the supervision of Dr. Michael Wheeler in 2003. Following this, Patrick spent time as a postdoctoral fellow in the group led by Dr. Patrik Rorsman, first in Sweden and then in the UK. In 2006, he was recruited back to Canada to set up his lab within the Alberta Diabetes Institute and Department of Pharmacology at the University of Alberta. In Edmonton, Patrick works with many outstanding team members at all career stages, who are focused on understanding the cell physiology of the islet cells. His team also runs the Alberta Diabetes Institute IsletCore, which is a biobank that provides research islets to groups across Canada and around the world.

The non-PI Leadership Awardee has been selected and will be announced and recognized during the Day 1 program of the National Meeting. Stay tuned!

Thank you to everyone who submitted nominations for these awards.

Thank you to our generous Sponsors!

GOLD LEVEL

SILVER LEVEL

Tuesday November 8, 2022

CIRTN-RF2IC National Meeting: SCIENTIFIC DAY

Glacier Ballroom – *Delta Hotel, Downtown Calgary*

7:00am – 8:00am BREAKFAST, Registration *Glacier Ballroom Foyer*

8:00am – 8:10am Welcome and Opening Remarks

Dr. Patrick MacDonald *(U Alberta)*, Dr. Christine Doucette *(U Manitoba)*

8:10am – 9:00am Keynote Lecture

Chair: Dr. Christine Doucette

Dr. Barbara Corkey, *Boston University*

"Basal Insulin Secretion: Causes and Consequences"

9:00am – 10:10am Session 1: Super Cool Technologies & Exciting Approaches to Study Islets

Chair: Dr. Erin Mulvihill

Talks (10 mins)

Design of an Islet-on-a-Chip Device to Dynamically Measure Insulin Secretion from Individual Islets
Yufeng Wang *(Rocheleau Lab)*

Using CD19 as a Selection Marker for the Scalable Production of Stem Cell Derived β-cells
Helen Huang *(Lynn Lab)*

Towards a Live-Cell Fluorescent Biosensor to Image β-cell Endoplasmic Reticulum Stress
Eric Floro *(Rocheleau Lab)*

A Dual-Reporter hESC Strain for Tracking Maturation of Stem-Cell-Derived β-Cells In Vitro
Carmen Bayly *(Lynn Lab)*

Variation in Membrane Excitability of Islet Cell Subpopulations is Linked to Marker Gene Expression
Birbickram Roy *(MacDonald Lab)*

Commercials (2 mins)

Investigating Alpha and β-Cell Phenotypes in Type 1 Diabetes
Theodore dos Santos *(MacDonald Lab)*

Do-it-yourself automated insulin delivery systems are non-inferior to commercial automated insulin delivery systems in glucose management among adults with type 1 diabetes: a real-world study
Zekai Wu *(Rabasa-Lhoret Lab)*

10:10am – 10:30am REFRESHMENT BREAK *Glacier Ballroom Foyer*

10:30am – 12:00pm Session 2: Islet Transplantation, Stem Cells & Other Novel Islet Therapies for Diabetes

Chair: Dr. Jenny Bruin

Talks (10 mins)

A Novel Small-Molecule Activator of Lyn Kinase for The Treatment of Type 1 Diabetes
Hui Huang *(Buteau Lab)*

Operational Tolerance of Murine Islet Allografts Co-transplanted with Cyclosporine A Eluting Microparticles
Puru Kuppan *(Pepper/Korbutt Labs)*

The Role of Altered Gut Microbiota in Type 1 Diabetes via Fecal Microbiota Transplantation
Rana Minab *(Verchere Lab)*

Mitigating Amyloid-associated Islet Transplant Failure with Pramlintide-expressing Human Embryonic Stem Cell-derived β-Cells
Saumadritaa Kar *(Verchere Lab)*

Co-culturing stem cell derived-β-cells and blood vessel organoids to improve vascularization of transplanted islets
Ekaterina Filatov *(Lynn Lab)*

Electrophysiological Characterization of Stem-Cell Derived β-Cells to Help Produce Clinically Relevant Cells for Transplant
Jasmine Maghera *(MacDonald Lab)*

Commercials (2 mins)

Effects of Notch Signalling on the Expansion of Human Stem Cell-Derived Pancreatic Cells
Amanda Oakie *(Nostro Lab)*

Exploring local immune modulation with rapamycin-eluting micelles to preserve islet graft function in mice
Jordan Wong *(Pepper Lab)*

A Small Molecule Activator of Lyn Improves the Outcomes of Islet Transplantation in Mice
Roozbeh Akbari Motlagh *(Buteau Lab)*

Layer By Layer Coating to Augment Graft Function
Kateryna Polishevska *(Pepper Lab)*

Reduced β-Cell *Ins2* has Sex-Specific Effects on Diabetes Incidence in NOD Mice
Natalie Nahirney *(Johnson Lab)*

12:00pm – 1:00pm LUNCH BREAK *Glacier Ballroom Foyer*

1:00pm – 3:00pm Session 3: All About the β-Cell
Chair: Dr. Rob Screaton

Talks (10 mins)

Mechanisms of β-cell Proliferation in Response to Insulin Resistance during Puberty
Clara Goubault *(Poitout Lab)*

Investigating the Relationship Between Pollutant Concentrations in Human Pancreas and Adipose Tissues and β-Cell Dysfunction
Myriam Hoyeck *(Bruin Lab)*

The role of prolactin signaling during metabolic stressors: multiple pregnancies and high fat diet.
Daniel Lee *(Huang Lab)*

Humbug is a novel vitamin K-dependent protein regulating calcium response in β-cells
Kevin Guo *(Ferron Lab)*

Proteomic Determinants of Nutrient-Specific Insulin Secretion Heterogeneity in Health and Disease
Jelena Kolic *(Johnson Lab)*

Optogenetic and pharmacological approaches to exploring the role of discrete Ca^{2+} pools in the initiation and transmission of Ca^{2+} waves across the islet
Luis Delgadillo *(Rutter Lab)*

A Short-Term High-Fat, Low-Carbohydrate Diet Mitigates Glucose Intolerance and Improves Insulin Secretion in Mice Carrying the HNF-1αG319S Variant
Taylor Morriseau *(Doucette Lab)*

Ketogenic Diet Intervention Increases *In Vivo* Incretin Response but Not GSIS
Cassie Locatelli *(Mulvihill Lab)*

Commercials (2 mins)

Cyp1a1/1a2 deletion protects female mice from high-fat diet induced glucose intolerance
Angela Ching *(Bruin Lab)*

	Single-cell RNA Sequencing Reveals a Role for Reactive Oxygen Species and Peroxiredoxins in Fatty Acid-induced Rat ß-cell Proliferation ***Alexis Vivoli*** *(Poitout Lab)* Glycine Receptor Activity in β Cells Is Downregulated in Type 2 Diabetes and After High Glucose Culture ***Amanda Schukarucha-Gomes*** *(MacDonald Lab)* The Impact of Inhibiting Prohormone Convertases on Human Pro-Islet Amyloid Polypeptide Processing ***Vriti Bhagat*** *(Verchere Lab)* Role of the Interaction Between NCK1 and PERK in Pancreatic β-Cell Function and Survival ***Laure Monteillet*** *(Estall Lab)* Individual Variability Dictates Cross-talk Between AhR and HIFα Signalling Pathways in Human Donor ***Noa Gang*** *(Bruin Lab)*
3:00pm – 3:20pm	REFRESHMENT BREAK *Glacier Ballroom Foyer*
3:20pm – 4:30pm	**Session 4: There's more to life than the β-cell, you know!** *Chair: Dr. Jen Estall*
Talks (10 mins)	FFA4 regulates insulin secretion via inhibition of somatostatin secretion from delta cells ***Laura Reininger*** *(Poitout Lab)* Master gene regulator Med15 is required for glucagon expression in adult mouse pancreatic alpha cells ***Samantha Mar*** *(Lynn Lab)* Exploring a Paradigm Shift in Islet Biology: The Emerging Role of α-cell Glucagon-Like Peptide-1 (GLP-1) ***Janyne Johnson*** *(Light Lab)* The Diabetes-Related Gly482Ser Polymorphism Affects PGC1A Stability and Glucose Metabolism ***Maria Galipeau*** *(Estall Lab)* Cisplatin Impairs Mitochondrial Function and Insulin Secretion in Mouse Islets ***Lahari Basu*** *(Bruin Lab)*
Commercials (2 mins)	Control of Post-prandial Intestinal Metabolism by Gut Hormone Receptor Signalling ***Nadya Morrow*** *(Mulvihill Lab)* Investigating the role of the HNF1aG319S variant in the metabolic response to fasting ***Manuel Sebastian*** *(Doucette Lab)* Discovering Novel Therapeutic Compounds for Type I Diabetes by Depleting Autoreactive T Cells ***Siyi He*** *(Lim Lab)*
4:30pm – 5:30pm	**Mentorship Awards & Lecture** *Chairs: Drs. Mathieu Ferron & Christine Doucette*
	PI Awardee: *Dr. Patrick MacDonald* (Sponsored by the Alberta Diabetes Institute) *"Some thoughts on Mentorship and CIRTN-R2FIC"* Non-PI Awardee: *To be announced in the award session* (Sponsored by the CR-CHUM) *$1000 honorarium*
7:00pm	Banquet Dinner at Saltlik *Cocktails & Hors D'oeuvres 7:00pm* *Dinner 8:00pm* 101 8th Ave SW SW (10-minute walk from the Delta Hotel)

Wednesday November 9, 2022

"ULTRA Scientists Day": Trainee Workshops

Joint with Diabetes Action Canada &CCHCSP/ENRICH

Glacier Ballroom – Delta Hotel, Downtown Calgary

Registered trainees only

7:00am – 8:00am	**Opening Ceremonies** *Elder Clement Leather, Eddie Wolf Child, and the Siksika Chief Crowfoot Elementary Drum Group*
8:00am – 9:00am	**BREAKFAST** (Registered ULTRA Trainees only), Registration *Glacier Ballroom Foyer*
9:00am – 9:10am	**Welcome and Land acknowledgement** *Susan Samuel (ENRICH) & André Tchernof (myROaD)* *Land Acknowledgement: Jon McGavock*
9:10am – 9:55am	**Patient Engagement in Research** ***Kathryn Birnie, Isabel Jordan***
9:55am – 10:40am	**Intersectionality Patient Engagement** ***Carolyn Shimmin***
10:40am – 10:55am	**REFRESHMENT BREAK** *Glacier Ballroom Foyer*
10:55am – 11:40am	**Implementation Science and Knowledge Translation** ***Gabrielle Zimmerman***
11:40am – 12:40pm	**LUNCH BREAK** *Glacier Ballroom Foyer*
12:40pm – 2:40pm	**Grant Writing Workshop** *"The Art of Writing a Knowledge Mobilization/Translation Plan"* ***Monika Castner***
2:40pm – 2:55pm	**REFRESHMENT BREAK** *Glacier Ballroom Foyer*
2:55pm – 3:40pm	**Mentorship Talk** *"How to Build Your Career"* ***Jennifer Yamamoto***
3:40pm – 4:25pm	**Conflict Resolution Talk** *"Having Difficult Conversations"* ***Lukas Neville***
4:25pm – 4:40pm	**Wrap-up & Invitation to Pub Scientifique** ***Susan Samuel & André Tchernof***
5:00pm – 8:00pm	**Pub night and Career Panel Discussion** (All Participants) *Shoe and Canoe – Delta Hotel* Light hors d'oeuvre will be served (Cash Bar)

Wednesday November 9, 2022

Networking Day & CIRTN-R2FIC Business Meeting

For PIs, Staff, and others NOT attending the ULTRA trainee day

10:00am – 2:00pm	Networking Activity: *Curling & Lunch* Garrison Curling Club 2288 - 47th Ave SW ***Shuttle bus pick up at 9:30am at the Delta Hotel – Meet in the lobby*
3:00pm – 5:00pm	CIRTN-RF2IC General Business Meeting Delta Hotel - *Room TBD* *Chair: Dr. Patrick MacDonald* Updates from all CIRTN-R2FIC committees. Agenda to be distributed.
5:00pm – 8:00pm	Pub Night and Career Panel Discussion (All Participants) *Shoe and Canoe Public House– The Delta Hotel* *Light hors d'oeuvres will be served (Cash Bar)*

SCIENTIFIC ABSTRACTS (by session)

Session 1: Super Cool Technologies & Exciting Approaches to Study Islets

Design of an Islet-on-a-Chip Device to Dynamically Measure Insulin Secretion from Individual Islets

Yufeng Wang, Romario Regeenes, Jonathan Rocheleau

University of Toronto, Toronto, ON.

Glucose-stimulated insulin secretion (GSIS) from pancreatic islets shows a biphasic pattern. Investigation of temporal dynamics and patterns of GSIS could give insights into mechanisms underlying β-cell function and dysfunction. Here, we present a novel microfluidic device to detect insulin secretion from individual islets on-chip through miniaturizing a fluorescence anisotropy immunoassay (FAIA). Insulin is co-secreted with c-peptide within ~5-10 min of glucose stimulation. Here we designed a c-peptide sensor with a fluorophore tagged to a synthetic c-peptide fragment and optimized assay parameters to maximize the sensitivity of the FAIA. Subsequently, a continuous-flow method was applied to monitor reaction kinetics to determine the equilibrium time of the assay. Using the maximum time required to reach equilibrium, we designed an islet-on-a-chip that captures the islet effluent and sufficiently mixes it with the reagents within the ~100 s residence time. This assay is downstream of the islet and leaves an ideal optical window to simultaneously image islet responses (e.g., Ca^{2+} activity). Ultimately, this device was used to measure c-peptide secretion from 4 individual mouse pancreatic islets simultaneously. The secretion detected on-chip shows the characteristic biphasic pattern and was further validated with pharmacological activators (ex. tolbutamide) for insulin secretion. In the future, the islet-on-a-chip could be coupled with other sensors, such as Ca^{2+} and oxygen consumption rate sensors to further probe β-cell metabolism.

Using CD19 as a Selection Marker for the Scalable Production of Stem Cell Derived β-cells

Luo Ting (Helen) Huang, Majid Mojibian, Francis C. Lynn

BC Children's Hospital Research Institute and Department of Cellular & Physiological Sciences, University of British Columbia, Vancouver, BC

Type 1 diabetes (T1D) is a disease characterized by autoimmune destruction of insulin-producing pancreatic β cells. Cadaveric islet transplantation is an effective treatment for T1D, but this procedure is limited by the scarce supply and inconsistent quality of islet tissue. Recently, stem cell-derived β-cells (SCβ-cells) emerged as a renewable and scalable alternative for cadaveric islets. We differentiate human embryonic stem cells (hESCs) as aggregates into SCβ-cells following a 6-stage, 20-day differentiation protocol. This protocol features a fluorescence-activated cell sorting (FACS) procedure to purify late-stage, immature SCβ-cells. However, FACS limits the scalability of SCβ-cells production, thus slowing the transition of SCβ-cells beyond the lab and into a clinical setting. Magnetic bead-based selection of SCβ-cells expressing a unique surface marker may obviate the need for FACS. CD19 is a B lymphocyte surface protein not expressed on SCβ-cells, for which there are clinical-grade microbeads that have been used for immunotherapies. In this study, we explored CD19 as a potential cell surface marker for the

purification of SCβ-cells. Using CRISPR/Cas9 technology, we knocked-in added-on a truncated form of CD19 downstream of the insulin coding sequence in hESCs. We differentiated the hESCs into SCβ-cells and magnetically enriched for CD19+ cells. Gene expression analysis confirmed that magnetic sorting enriched for INS-expressing cells over other endocrine cell types. Sorted SCβ-cells also showed a trend of increased c-peptide secretion in response to high glucose. We expect this selection method to generate a therapeutic dose of 1 million aggregates per differentiation, facilitating the transition of SCβ-cells into clinical settings.

Towards a Live-Cell Fluorescent Biosensor to Image β-cell Endoplasmic Reticulum Stress

Eric Floro, Alex M. Bennett, and Jonathan V. Rocheleau

University of Toronto, Toronto, ON.

Endoplasmic Reticulum (ER) stress is caused by an accumulation of misfolded proteins. When unmitigated, it results in metabolic dysfunction and apoptosis, and is a key mechanism responsible for the progression of β-cell failure leading to Type 2 Diabetes. In response to protein misfolding, a transmembrane stress-sensing protein called IRE1α can activate the Unfolded Protein Response (UPR). In the early stages of the UPR, protein translation is attenuated to allow for refolding and repair. If unsuccessful, degradation of protein and RNA begins, and apoptosis may be triggered. To measure the dynamics of ER stress in living β-cells, we are developing a genetically encoded sensor based on IRE1α. This sensor uses a fluorescent protein-tagged IRE1α, which homo-oligomerizes to dimeric and tetrameric conformations under early and chronic ER stress, respectively. These conformational changes can be quantified through changes in the fluorescent polarization ratio of the sensor. To validate this sensor, we have demonstrated the sensor responds to chemical and physiological (high glucose) stress induction in a rat β-cell line (INS-1E) and mouse islets. Preliminary data suggest that UPR-associated XBP1 mRNA splicing is highly correlated with sensor response. Furthermore, individual cells with the highest sensor response (correlating to late-stage UPR) show elevated levels of pro-apoptotic protein TXNIP. These data are consistent with a working sensor that can be used to investigate ER stress dynamics in β-cells.

A Dual-Reporter hESC Strain for Tracking Maturation of Stem-Cell-Derived β-Cells *In Vitro*

Carmen L. Bayly and Francis C. Lynn

BC Children's Hospital Research Institute, University of British Columbia, Vancouver, BC.

Type 1 diabetes (T1D) is characterized by a loss of β-cell function, namely the secretion of insulin in response to blood glucose. Islet transplantation removes the need for dependence on insulin pumps or daily injections, but demand far exceeds supply. Human embryonic stem cell-derived β-cells (SC- β-cells) provide a promising, more scalable approach to treating T1D. However, current *in vitro* differentiation procedures yield immature SC-β-cells, which express less insulin and release insufficient quantities in response to glucose. These SC-β-cells mature post-transplantation, inviting further research to identify ligands and small molecules that could be used to achieve more complete maturation *in vitro*. To this end, we describe a dual-reporter hESC strain developed for evaluating the effects of culture methods and additives on SC-derived β-cell maturation *in vitro*. This strain carries fluorescent protein reporters for tracking insulin and islet amyloid polypeptide (IAPP) expression, which are used to indicate SC-β-cell identity and maturation respectively. We plan to show that SC-β-cells expressing both reporters are

phenotypically and functionally more mature than those expressing insulin alone, as measured by maturation marker expression and glucose stimulated insulin secretion (GSIS). With these indicators of maturity established, this strain can be used as a tool to evaluate differentiation procedures for improved SC-β-cell maturation.

Variation in Membrane Excitability of Islet Cell Subpopulations is Linked to Marker Gene Expression

Birbickram Roy, Austin Bautista, and Patrick E MacDonald

Department of Pharmacology and Alberta Diabetes Institute, University of Alberta, Edmonton, AB

Pancreatic α and β cells are functionally heterogeneous. Using linked electrophysiological profiling and single-cell RNA sequencing (patch-seq) in human islet cells, we previously showed heterogeneity of Na^+ channel currents amongst sub-populations of α and β cells. This suggests that islet cell sub-populations differ in their inherent excitability, although this has not been tested directly. To address this, we investigated action potential firing properties of dispersed human islet cells coupled with a single-cell RT-qPCR panel for thirteen cell-type and sub-population markers. Our results show that significant difference in membrane excitability is associated with heterogenous expression of transcriptomic markers such as *LOXL4, ARX, and FEV* in a-cells, and *RBP4, MAFA, and NKX 6.1* in β-cells. We found that α and β-cells from non-diabetic (ND) donors in general fire action potentials (APs) at higher frequencies compared to cells form type-2 diabetic (T2D) donors. Among a-cell subpopulations, the ND *FEV*+ cells (ND-a_{FEV+}) required higher current injection to evoke APs, showed significantly increased AP half-width, and reduced K^+-currents compared to ND-a_{FEV-} cells, indicating lower membrane excitability. These differences were lost in T2D-a_{FEV} subpopulations. In β-cell subpopulations, both ND- and T2D-b_{MAFA+} cells show significantly reduced AP frequency compared to b_{MAFA-} cells, whereas increased membrane excitability was observed in ND-b_{RBP4+} cells compared to ND-b_{RBP4-} cells. We are currently corroborating our findings using live human pancreatic slices, allowing electrophysiological characterization, and mapping of the islet cell subpopulations in their native microenvironment.

Investigating Alpha and β-Cell Phenotypes in Type 1 Diabetes

Theodore dos Santos[1], XiaoQing Dai[1], Cara Ellis[1], Joan Camunas-Soler[2], Austin Bautista[1], Patrick E. MacDonald[1]

Alberta Diabetes Institute, University of Alberta, Edmonton, AB, Canada[1], Oxford Centre for Stanford University School of Medicine, Stanford, CA, United States of America[2]

Background: In Type 1 Diabetes (T1D), insulin producing β-cells are destroyed by the immune system, resulting in a lifelong dependence on insulin therapy. Currently, there is considerable heterogeneity in the pathology, genetics, and response to therapies in T1D[1]; however, little research has focused on studying how α-cells and surviving β-cells behave in T1D. Previously[2], we studied α-cell heterogeneity in Type 2 Diabetes and discovered that only a sub-set of α-cells expressing elevated stem-cell markers were malfunctioning. In this project, we apply a similar approach to investigate the behaviour of α- and β-cells in T1D.

Methods: Our lab uses the novel technique of Patch-Seq, allowing us to record the electrical behaviour (i.e., function) of a cell, and then sequence its transcriptome (i.e., gene expression); thus, permitting us to link a cell's gene expression with its behaviour. Uniform Manifold Approximation and Projection (UMAP) approaches were used to analyze the transcriptome of

T1D α- and β-cells, in the context of control α- and β-cells from matched (age, sex, and BMI) Non-Diabetes (ND) donors. Previously developed machine learning methods were applied to the patching data to empirically compare modelling scores between α- and β-cells in T1D and ND.

Results: Compared to matched ND controls, T1D α-cells demonstrate significantly altered electrical properties, whilst β-cells were comparable, at 5mM glucose conditions (euglycemia). Of mention, capacitance values were elevated in T1D α-cells, suggesting increased exocytotic activity. However, AI modelling revealed a significant decrease in both α- and β-cell scores in T1D, suggesting that when analysed holistically, overall electrical behaviour is altered. Lastly, our UMAP analysis of gene expression revealed α-cell subtypes that were enriched in T1D.

Conclusions: Our results demonstrate that in T1D, α-cells demonstrate altered electrical identity and gene expression profiles. On the other hand, β-cells transcriptomes in T1D are comparable to ND, as well as their electrical properties when analyzed independently. However, when modelled holistically by our AI, T1D β-cells possess electrical identities that are significantly altered compared to ND, and this finding requires further investigation. Our next step is to identify which genes are linked to the altered behaviour we observe in T1D.

Do-it-yourself automated insulin delivery systems are non-inferior to commercial automated insulin delivery systems in glucose management among adults with type 1 diabetes: a real-world study

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BACKGROUND: We aim to compare Health Canada approved commercial automated insulin delivery (AID) systems with unregulated open-source do-it-yourself (DIY)-AID systems for glucose management among adult people with type 1 diabetes (PWT1D) in real-life conditions.

METHODS: Planned interim analysis of a prospective non-inferiority, non-randomized, parallel-cohort study involving 45 non-pregnant adult PWT1D having used an AID for ≥3 months and living in Canada: 15 DIYAID and 30 commercial AID users, meeting a prespecified ratio of 1(DIY):2 (Commercial); 55.6% females, mean age 48.0±14.2 years old with mean diabetes duration of 29.9±15.3 years and HbA1c of 6.7±0.7%. Participants continued using their system as per usual. Four weeks' data from an additional blinded CGM (Dexcom G6) was used to assess effectiveness [Primary outcome: 24-hour time in range% (TIR%)].

RESULTS: DIYAIDs were non-inferior to commercial AIDs regarding the 24h TIR% (82.0±8.1% vs. 73.1±9.0%, mean difference 9.0% [95% CI 3.4% to 14.5%], P<0.001 for non-inferiority [non-inferiority margin 5%]), even after adjusting for confounding factors (Figure 1). 24-hour outcomes including mean glucose (7.4±0.8% vs. 8.5±0.9%, P<0.001) and hyperglycemia (>10.0 mmol/L: 14.1±8.4% vs. 25.1±9.6%, P<0.001) were better in DIYAID than commercial AID users while percentage in hypoglycemia was higher (3.8±2.5% vs. 1.8±1.1%, P<0.001) when using DIYAID. The benefits of DIYAID were mostly attributed to the daytime period.

CONCLUSION: In a real-world setting, DIYAID was non-inferior to commercial AIDs for TIR% with an increase yet within recommended range in time in hypoglycemia among adult PWT1D.

Session 2: Islet Transplantation, Stem Cells & Other Novel Islet Therapies for Diabetes

A Small Molecule Activator of Lyn Improves the Outcomes of Islet Transplantation in Mice

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Islet transplantation can achieve insulin independence in individuals with type 1 diabetes. However, islets derived from multiple donors are often required, and functional β-cells are lost early after transplantation. There is thus a need for strategies to improve graft survival and function. Our lab has recently characterized Lyn as a critical regulator of β-cell proliferation and survival. We herein sought to test the hypothesis that pharmacological activation of Lyn improves the outcome of islets transplantation in mice.

Male BALB/C islets were isolated and transplanted (marginal mass of 125 islets) into syngeneic diabetic mice recipients under the left kidney capsule. Recipients were thereafter injected intraperitoneally once daily with a specific activator of Lyn (MLR-1023) or vehicle for 7 days. Glucose tolerance was performed on days 8 and 28 post-transplant. The graft-bearing kidneys were also harvested for immunohistochemical analysis.

A brief 7-day treatment with MLR-1023 was sufficient to stimulate β-cell proliferation in islet recipients. However, β-cell mass was not significantly altered, due to inter-individual variations. MLR-treated mice also displayed improved graft vascularization compared to controls. Remarkably, these results translated into better glucose tolerance in the MLR-treated group compared to controls at day 8, concomitantly with increased insulin secretion. However, the effects of MLR-1023 dissipated 21 days after drug withdrawal.

In summary, MLR-1023 could be used in clinical islet transplantation to reduce the islet mass that is required to achieve insulin independence or to accelerate the time to normoglycemia.

Operational Tolerance of Murine Islet Allografts Co-transplanted with Cyclosporine A Eluting Microparticles

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β-cell replacement therapy is a proven strategy to restore glycemic control, reduce hypoglycemic awareness, and stabilize HbA1c for a subset of patients with type 1 diabetes. However, the absence of an effective strategy to prevent islet allograft rejection and recurrent autoimmunity restricts patient inclusion and durable insulin independence. Herein, we explore the utility of islet graft localized cyclosporine A (CsA) delivery via co-transplanted drug-eluting poly(lactic-co-

glycolic acid) (PLGA) microparticles to attenuate allograft rejection. Diabetic BALB/c mice co-transplanted with syngeneic islets and 4 mg of CsA microparticles (10 mg/kg of CsA) demonstrated similar engraftment and functional profiles as islet alone recipients. Subsequently, as a monotherapy, graft localized CsA release significantly delayed islet allograft (diabetic C57BL/6 mice received BALB/c islets) rejection compared to controls ($p<0.05$). Over 50% (6 of 11) of recipients receiving localized CsA microparticles and acute posttransplant systemic CTLA4-Ig therapy displayed prolonged allograft survival, >214 days, compared to 25% (2 of 8) of recipients receiving CTLA4-Ig therapy alone. Grafts from CsA microparticle recipients exhibited reduced presence of immune cells ($CD4^+$, $CD8^+$ $CD68^+$), and reduced mRNA expression of proinflammatory cytokines and chemokine compared to islet alone grafts. Long-term islet allografts contained robust $insulin^+$ and intra-graft $FoxP3^+$ cells. Rapid rejection of third party skin grafts (C3H) in islet allograft recipients suggests localized CsA + acute CTLA4-Ig induces operational tolerance. Graft localized immunosuppression could promote an immune protective transplant niche for allogeneic islets and reduce the reliance on systemic immunosuppression.

The Role of Altered Gut Microbiota in Type 1 Diabetes via Fecal Microbiota Transplantation

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The gut microbiome is a potential driver of autoimmunity in type 1 diabetes (T1D). A recent clinical study showed that in new-onset T1D, autologous fecal microbiota transplantation (FMT) preserved β-cell function for 12 months (de Groot *et al*, 2020). To gain insight into the mechanism underlying this protective effect, we are assessing the impact of human FMT in the NOD mouse model of T1D. We hypothesize that FMT from healthy humans will delay diabetes onset and improve β-cell function. Human feces are transplanted into 6-week-old NOD mice pre-treated with antibiotics to eliminate native microbiota. Blood glucose is monitored and following development of diabetes or at 30 weeks of age, microbiota and pancreas will be assessed by metagenomic analysis and histology. Quantification of β-cell mass and inflammatory cell infiltration within the pancreatic islets will be done by immunostaining. In a pilot study, we administered FMT from a healthy human (or saline control) to pre-diabetic NOD mice. Preliminary metagenomic analysis indicates successful reconstitution of the murine gut with human microbiota at 2- and 4-weeks post-FMT. In this preliminary cohort, 7 of 10 human FMT recipients and 5 of 10 saline recipients had turned diabetic by 24 weeks, suggesting no clear difference in diabetes onset. Next, FMT with feces from T1D individuals into NOD mice will be performed. Our goal is to elucidate the role of the microbiome in T1D pathogenesis in the hope of developing microbiome-based therapies for T1D.

Mitigating Amyloid-associated Islet Transplant Failure with Pramlintide-expressing Human Embryonic Stem Cell-derived β-Cells

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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 considerable 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 optimization. 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. Preliminary 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 further 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 improved fasting blood glucose and a detectable C-peptide response at 6-weeks with a glucose tolerance test, compared to mice receiving no transplant. Our data suggest pramlintide expression does not impact SC-β cell maturation and may control hyperglycemia in immunodeficient diabetic mice. Future studies will focus on evaluating efficacy of pramlintide-expressing cells in transplant models.

Co-culturing Stem Cell-Derived β-cells and Blood Vessel Organoids to Improve Vascularization of Transplanted Islets

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Microvasculature in human pancreatic islets impacts β-cell insulin secretion, β-cell mass, and proliferation. Additionally, the lack of vascularization of transplanted islets leads to reduced graft function and survival. The generation of β-cells from stem cells has potential as being a source for transplants as a therapy for type 1 diabetes; however significant cell death occurs following transplantation. As such, we hypothesize that the vascularized sc-β-cells will improve insulin secretion in response to glucose and will increase macrovascularization of the graft, prolonging its survival. Prior to transplantation, sc-β-cells and sc-blood vessel organoids will be co-cultured so that microvasculature can penetrate the clusters of sc- β-cells. Markers of maturation (*e.g. Ins*, *Mafa*, *Ucn3*, *Iapp*) and proliferation (*e.g. Mki67*, *Itgb1*, *Myc, Ccnd3*) will be monitored over time using qPCR, flow cytometry and immunostaining with confocal microscopy, while observing any morphological changes. To date, our gene expression data shows improved expression of some maturation markers when culturing sc- β-cells in sc-blood vessel organoid media. Once sc-β-cell clusters are successfully vascularized, we will use single cell RNA-sequencing to uncover unexpected cell populations that arise during co-culture of sc-β-cells and sc-blood vessel organoids, and we will transplant the co-cultured cells into STZ-treated immunodeficient mice.

Electrophysiological Characterization of Stem Cell-Derived β-Cells to Help Produce Clinically Relevant Cells for Transplant

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Background: Although significant progress has been made in treating type 1 diabetes with islet transplantation[1], new cell sources are needed since organ donors remains limited. Stem cell-derived β-cells (SCβ-cells) offer a promising alternative for cell replacement therapy; however, key characteristics displayed by mature primary human β-cells, such as glucose-regulated excitability and insulin secretion via the exocytosis of secretory granules, remain blunted in SCβ-cells, reflecting immaturity after cellular differentiation[2].

Methods: We seek to characterize the electrical and secretory machinery responsible for regulation of insulin secretion, comparing SCβ-cells and primary human β-cells. Single-cell patch-clamp electrophysiology was used to assess the cell size, the activity of ion channels involved in action potential firing, and the fusion of secretory vesicles with the plasma membrane.

Results: We found that that stage 6 SCβ-cells (n=42) are significantly ($p<0.0001$) larger in cell size than primary β-cells (n=51 from 24 donors), having a 2.47-fold larger cell membrane capacitance. The SCβ-cells display a 9.15-fold larger ($p<0.0001$) exocytosis compared with primary β-cells, even when normalized to cell size. This increased exocytosis is paralleled by increased voltage-activated Na^+ currents (2.61-fold increased, $p<0.0001$) and Ca^{2+} (4.61-fold increased, $p<0.0001$), the latter being mediated mostly by L-type channels.

Conclusions: These findings suggest that, although SCβ-cells act differently compared with primary β-cells, the mechanical machinery responsible for excitability and exocytosis is intact and functional by stage 6 of differentiation. Future studies will examine metabolic differences between derived SCβ-cells and primary β-cells that reduce glucose sensing and insulin release upstream of this secretory machinery.

Effects of Notch Signalling on the Expansion of Human Stem Cell-Derived Pancreatic Cells

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Human pluripotent stem cell (hPSC)-derived β-like cells are a promising cell source for restoring insulin in people living with type 1 diabetes. However, current differentiation protocols produce a low β-like cell yield insufficient for clinical transplantation. The Notch signalling pathway regulates early pancreatic cell expansion and endocrine commitment *in vivo*. This study examines the role of Notch during hPSC differentiation to pancreatic cells and its potential to induce pancreatic cell expansion. Gene expression analyses of Notch signalling components throughout differentiation revealed increased expression of receptors, ligands, and the Notch-associated transcription factor HES1 in early $PDX1^+$ pancreatic cells. To examine whether Notch activation promoted cell expansion, $PDX1^+$ cells were reseeded on control OP9 stromal cells or on OP9 cells expressing the Notch ligands delta-like ligand (DLL) DLL1 or DLL4. Cell numbers were quantified throughout

co-culture differentiation and samples were analyzed using flow cytometry analysis. Findings demonstrated that PDX1$^+$ cells cultured on all OP9 conditions were able to expand following passage and maintained PDX1$^+$ pancreatic cell fate. Despite the increase in cell numbers, we did not detect any significant differences in the frequency of Ki67$^+$/PDX1$^+$ cells amongst the different treatment groups, which may indicate that OP9 cells support PDX1$^+$ cell survival rather than proliferation. All OP9 co-cultures were able to differentiate to β-like cells and produced 2-3 times more total cells than control differentiations. Further experiments will focus on the mechanisms through which OP9 cells support PDX1$^+$ cell expansion.

Exploring local immune modulation with rapamycin-eluting micelles to preserve islet graft function in mice

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Islet transplantation is an effective means for a subset of people with type 1 diabetes to achieve insulin independence; however, lifelong immunosuppression required to subvert the immune response remains a barrier in patient inclusion. Herein, we explore the use of a localized drug delivery system to preserve islet allograft function, reducing the need for toxic systemic immunosuppression. Rapamycin (rapa) was encapsulated in Food and Drug Administration-approved poly(lactide-co-glycolide) micelles. Sustained *in vitro* release was observed over 35 days and, moreover, human islets co-cultured with rapa-micelles for 24 hours had comparable functions to controls when assessed for mitochondrial potency and glucose-stimulated insulin secretion. Conversely, human islets from the same donor incubated in 25 nM rapa had blunted glucose-stimulated respiration. Syngeneic islets co-transplanted with 0.2 mg/kg rapa-micelles (n=8) into diabetic mice demonstrated partial graft function with 38% remaining euglycemic for 36 days posttransplant, while all 0.1 mg/kg rapa-micelle recipients (n=3) remained euglycemic with similar glucose tolerance to controls. Preliminary results of fully major histocompatibility complex-mismatch murine islet allografts demonstrated prolonged function when co-transplanted with 0.1 mg/kg rapa-micelles compared to empty micelles. At the endpoint, grafts are collected and stained for endocrine and immune cell markers (CD4$^+$, CD8$^+$, FOXP3$^+$). Immunohistochemistry and genetic profiling will be completed for our ongoing subcutaneous porcine allotransplant model testing rapa-micelles. Collectively, localized drug delivery has the potential to alter the immune environment, protect grafts, and serve as a safe adjuvant approach in clinical islet transplantation.

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

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It is recently known that individuals with long-standing type 1 diabetes (T1D) still have surviving functional β-cells. Thus, strategies to promote β-cell protection/replication should be used in the adjuvant therapy of T1D. Our lab has extensively characterized Lyn kinase as a critical regulator of β-cell mass. We herein hypothesized that pharmacological activation of Lyn could stimulate β-cell regeneration and improve glucose control in animal models of T1D.

A short treatment of 7-days with MLR1023, a specific activator of Lyn, was sufficient to improve glucose tolerance, and to induce a 2-fold increase in β-cell mass in diabetic NOD mice. The number of PCNA-positive and TUNEL-positive β-cells highlighted the contribution of both proliferation and apoptosis to β-cell mass expansion. Replication of α-cells and ductal cells was not altered by MLR1023 treatment, limiting the possibility of off-target effects. Several morphological parameters of "islet health", including insulitis scores, were improved by MLR1023. Our results were recapitulated in streptozotocin-diabetic mice, used as a second model of T1D. Conversely, mice with β-cell-specific deletion of Lyn did not respond to MLR1023, confirming the implication of Lyn and the direct action of MLR1023 on β-cells. In isolated human islets, MLR1023 significantly increased β-cell proliferation, and prevented apoptosis induced by cytokines and glucotoxicity, thereby suggesting that MLR1023 could exert similar beneficial actions in humans with T1D.

In conclusion, we suggest that small molecule activators of Lyn could be used to delay or cure T1D.

Layer By Layer Coating to Augment Graft Function

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Transplantation of pancreatic islet represents a proven therapeutic strategy to restore physiologic glycemic control for patients with T1DM who suffer from life-threatening severe hypoglycemia unawareness. However, limiting factors prevent islet transplantation from replacing insulin therapy, including donor shortage and lifelong immunosuppression. Islet encapsulation has the potential to reduce the immune reaction. We hypothesize that conformal islet coating with poly(N-vinylpyrrolidone) (PVPON) and tannic acid (TA) PVPON/TA will enhance the engraftment efficacy of human islet xenografts as well as murine islet allografts.

Human and murine islets were coated with PVPON and TA to form 3.5 deposit bilayers. Confirmation that PVPON/TA does not hinder islet function was examined by the *in vitro* function of coated and non-coated human islets and confirmed *in vivo* function by transplanting these human islets into diabetic immunodeficient Rag-/- mice. Subsequently, the immunoprotective properties of PVPON/TA coating were examined by transplanting coated and non-coated islets in our well-established murine islet allograft model.

Both control and coated islets exhibited similar results in all *in vitro* assays performed. Human islet recipients transplanted with PVPON/TA coated islets reversed diabetes, proving this coating technique is non-toxic. Data from allograft recipients demonstrate that PVPON/TA coating reduces intragraft inflammation and delays allograft rejection. The present study demonstrates that PVPON/TA coated islets retain their *in vitro* and in *in vivo* functional potency. This transplant approach has the potential to reduce post-transplant inflammatory responses, high possibility of translation to clinical investigation, improve islet allograft survival, and eliminate the need for toxic systemic immunosuppression.

Reduced β-Cell *Ins2* has Sex-Specific Effects on Diabetes Incidence in NOD Mice

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As the body's only source of circulating insulin, β-cells have a high protein production demand that can cause endoplasmic reticulum (ER) stress, even under normal conditions. ER stress in β-cells has been proposed to contribute to the initiation and progression of type 1 diabetes (T1D), via the generation of neo-autoantigens and the increased fragility that predisposes to β-cell death. The human *INS* gene accounts for 10% of the heritable T1D risk. Our recent analysis of single cell RNA sequencing data from human β-cells has showed that T1D risk is associated with a paradoxical increase in insulin production and markers of ER stress. In the present study, we propose to directly test the role of insulin production in the pathogenesis of T1D. We hypothesize that a mild reduction of insulin production may alleviate ER stress and delay the onset of diabetes. Mice have a rodent-specific β-cell insulin gene (*Ins1)* in addition to the ancestral human homolog for insulin, *Ins2,* which exists in the brain, thymus, and β-cells. Insulin gene knockouts have previously been shown to alter diabetes progression in non-obese diabetic (NOD) mice, but the consequences of reduced *Ins2* expression specifically in β-cells has not been tested. We developed a NOD mouse model with Cre expressed on the endogenous *Ins1* locus (*Ins1*$^{Cre/wt}$) for β-cell specificity and crossed it with floxed *Ins2* alleles to generate either full knockout (*Ins2*$^{flox/flox}$) or heterozygous (*Ins2*$^{wt/flox}$) mutants. We noted a sex-specific response to this genetic manipulation. Male *Ins2* knockout mice (*Ins1*$^{cre/wt}$;*Ins2*$^{flox/flox}$) developed early diabetes at 6 weeks of age, likely due to insulin insufficiency, yet female littermates of the same genotype were protected from diabetes up to 47 weeks. The *Ins2* reduction (*Ins1*$^{cre/wt}$;*Ins2*$^{wt/flox}$) was protective for male mice up to 47 weeks but only up to 25 weeks for females. Importantly, the control expression of Cre (*Ins1*$^{cre/wt}$*;Ins2*$^{wt/wt}$) showed a protective effect for both males and females. Additional studies demonstrated Cre-mediated protection beyond that which was observed in the *Ins1*^{neo} knockout allele. Ongoing studies to elucidate the β-cell specific role of *Ins2* are using time-controlled delivery of *Ins1*-Cre with a pancreas-selective adeno-associated virus (AAV8-*Ins1*-Cre). Though still early in this phase of the project, we have shown effective gene deletion with this method by repeating the early-onset diabetes phenotype in male *Ins2*$^{flox/flox}$. Under these conditions of early-adult insulin reduction, we plan to study autoimmunity and β-cell stress to further elucidate the contribution of insulin-production stress to T1D pathogenesis.

Session 3: All About the β-Cell

Mechanisms of β-cell Proliferation in Response to Insulin Resistance during Puberty

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Puberty is associated with a transient period of insulin resistance. In overweight children, insulin resistance persists after puberty leading to an increased risk of type 2 diabetes in adulthood. How β-cells adapt to pubertal insulin resistance is unknown.

The aim of this study was to identify the mechanisms underlying β-cell proliferation during puberty.

In rats, the percentage of proliferative β-cells transiently increased to 1.5+/-0.5 % during puberty compared to 0.3+/-0.1 % before puberty in both sexes (n=3-5, p<0.001), as assessed by Ki67 and insulin staining of pancreatic sections. Out of 7 human pancreatic sections, Ki67-positive β-cells were only detected in the 3 samples from pubertal donors. Exposure of isolated rat and human islets to 10% pubertal serum for 72h promoted β-cell proliferation compared to weaning serum (rats: 3.8+/-1.3 vs. 2.3+/-0.8 %, n=9, p<0.05; humans: 0.04+/-0.01 vs 0.10+/-0.04 %, n=6-9, p<0.01), as assessed by flow cytometry for EdU and C-peptide or insulin. mRNA expression of the pro-proliferative isoform of the serotonin receptor HTR2b was increased in islets during puberty. The HTR2b inhibitor SB204741 (35 mM) blocked the increase in β-cell proliferation induced by pubertal serum in islets (3.6+/-1.4 vs. 6.0+/-2.3 %, n=7, p<0.05). Similarly, treatment of prepubertal rats with SB204741 (1 mg/kg/d for 12 days) inhibited β-cell proliferation (1.6+/-0.8 vs. 3.0+/-1.3 % in vehicle-treated animals, n=4-5, p<0.05).

We conclude that β-cell proliferation during puberty is mediated by serotonin signaling in islets and a circulating factor that remains to be identified.

Investigating the Relationship Between Pollutant Concentrations in Human Pancreas and Adipose Tissues and β-Cell Dysfunction

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Epidemiological studies consistently report an association between circulating concentrations of persistent organic pollutants and increased diabetes risk in humans. We hypothesize that lipophilic pollutants accumulate in human pancreas tissue and cause β-cell injury. To test this hypothesis, we measured the concentration of persistent pollutants from 3 chemical classes (dioxins, polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs)) in pancreas and adipose tissues from 30 organ donors using GC-MS/MS. Our goal is to determine whether pollutant concentrations in adipose and/or pancreas correlate with islet transcript profiles, dynamic regulated insulin secretion phenotypes, or other donor characteristics. Thus far, our preliminary data in 19 donors show that these pollutants are consistently detectable in human pancreas, and for some donors, at higher concentrations than in adipose. We also found that pancreatic dioxin concentrations are positively correlated with islet levels of *CYP1A1*, a xenobiotic metabolism enzyme known to be upregulated by dioxin exposure. These data confirm that pollutants are accumulating in human pancreas, which could directly impact islet health. Interestingly, concentrations of dioxins, PCBs, and OCPs in pancreas are negatively correlated with genes involved in insulin secretion (e.g. *GP6PC2*) and β-cell development/identity (e.g. *NKX2.2*). These data support our hypothesis that pollutant exposure can lead to impaired β-cell function, which we will further assess in the next steps of our analyses.

The Role of Prolactin Signaling during Metabolic Stressors: Multiple Pregnancies and High Fat Diet

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β-cell adaptation in response to metabolic stressors is an important aspect of preventing diabetes. Our lab has previously shown that prolactin receptor (Prlr) signaling is required for normoglycemia during pregnancy. This study aimed to elucidate the role of Prlr signaling in the adaptation of β-cells to multiple pregnancies followed by high fat diet (HFD). Female transgenic mice with an inducible, β-cell-specific homozygous deletion of Prlr (βPrlr$^{-/-}$) were set up for pregnancies and after delivering the second litter, placed on a HFD (60% calories from fat) for 12 weeks. After 2 pregnancies, a difference was observed during intraperitoneal but not oral glucose tolerance tests. After 12 weeks of HFD, bPrlr$^{-/-}$ mice exhibit glucose intolerance where the area under the curve (AUC) during an oral glucose tolerance test: βPrlr$^{+/+}$: AUC=1070$\pm$268, bPrlr$^{-/-}$: AUC= 1419$\pm$378 (p=0.00007). *In vivo* insulin secretion at 10 minutes was decreased βPrlr$^{+/+}$: 4.2$\pm$1.8-fold, bPrlr$^{-/-}$: 2.5$\pm$1.1-fold from baseline (p=0.04). No difference during *ex vivo* glucose-stimulated insulin secretion, however, total insulin content was greater in βPrlr$^{+/+}$: 147196$\pm$15179 ng compared to bPrlr$^{-/-}$: 75007$\pm$20808 ng (p=0.001). Finally, morphometrics revealed a difference in β-cell mass βPrlr$^{+/+}$: 3.06$\pm$0.45 mg, bPrlr$^{-/-}$: 2.25$\pm$0.22 mg (p=0.01) but no difference in β-cell proliferation. No difference in insulin tolerance between genotypes was observed. These results suggest that Prlr signaling in β-cells is required to meet the cumulative demands of multiple pregnancies and HFD. We now aim to investigate the molecular mechanisms that govern these differences.

Humbug is a Novel Vitamin K-dependent Protein Regulating Calcium Responses in β-cells

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Clinical studies have recently implicated vitamin K (VK) in the pathophysiology of diabetes, but the underlying molecular mechanism remains unknown. VK is an essential cofactor for gamma-carboxylase (GGCX), which converts glutamic acid (Glu) into gamma-carboxyglutamic acid (Gla) residues in secreted proteins within the endoplasmic reticulum (ER). Our data indicate that GGCX and carboxylation are present in pancreatic β-cells and that β-cells lacking GGCX fail to adapt their insulin secretion in response to glucose in the context of age-related insulin resistance or diet-induced β-cell stress. The aim of this study was to identify and characterize the gamma-carboxylated protein(s) mediating the effect of VK in β-cells.

Using proteomics, we identified humbug as a novel gamma-carboxylated ER-resident protein. Biochemical and histological experiments established that humbug is expressed and carboxylated in β-cells in vivo. Deletion and mutagenesis studies showed that several Gla residues are localized in a luminal "Glu-rich domain" of humbug. Calcium overlay assays demonstrated that carboxylation of humbug increases its calcium binding capacity. In HEK293 cells, carboxylated humbug dampens store-operated calcium entry (SOCE) and reduces the formation of STIM1-Orai1 membrane puncta that are necessary for SOCE. Conversely, *Ggcx*-deficient β-cells lacking carboxylated humbug showed increased SOCE and elevated basal calcium. This deregulation in calcium flux in the absence of carboxylated humbug, may explain the failure of the *Ggcx*-deficient β-cells to adapt their insulin secretion in response to metabolic

stress. Altogether, our work provides the basis for a potential molecular and cellular mechanism by which VK may protect from diabetes.

Proteomic Determinants of Nutrient-Specific Insulin Secretion Heterogeneity in Health and Disease

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While glucose is the main driver of acute insulin secretion, other dietary nutrients can also stimulate insulin release. However, the degree to which non-carbohydrate substrates release insulin and the individual-to-individual variation is poorly understood. We report a large-scale survey of human islet insulin secretion in response to model macronutrient stimuli, alongside proteomic analysis to a depth of >7800 proteins, gross-donor characteristics, and islet-isolation parameters. Islets from 138 donors, 16 with type 2 diabetes, were from the Alberta Diabetes Institute IsletCore. Dynamic insulin secretion analyses in response to glucose (15 mmol/L), leucine (5 mmol/L), and oleate/palmitate (1:1 mix, 1.5 mmol/L) showed significant heterogeneity between donors. We identify subgroups of donor islets that are hyperresponsive to either proteins or lipids and show that these responses are correlated to significant changes in abundance of proteins involved in mitochondrial signaling and/or fatty acid metabolism using high-depth quantitative mass spectrometry (DIA-PASEF). We also show that islets from donors with type 2 diabetes have lower overall insulin responses to glucose and fatty acids, but not amino acids, and find 640 differentially abundant proteins ($p<0.05$) between the two groups. Importantly, there were few significant correlations between insulin responses and donor characteristics and islet isolation parameters, pointing to genuine biological variability rather than technical artifacts. This study represents the most detailed analysis of the human islet proteome and reveals the underlying mechanisms of nutrient response and type 2 diabetes.

Optogenetic and Pharmacological Approaches to Exploring the Role of Discrete Ca^{2+} Pools in the Initiation and Transmission of Ca^{2+} Waves Across the Islet.

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Recently, two subpopulations of β-cells with a disproportionate control overt islet Ca^{2+} dynamics have been identified. Using Ca^{2+} imaging, pharmacology and optogenetics, we (Johnstone et al, 2016) mapped a subpopulation of β-cells which serve as highly-connected “hub” cells to regulate the islet glucose response. We subsequently identified a further subpopulation termed “leader” β-cells (Salem, Delgadillo, et al, 2019). Whilst photo-ablation of Zebrafish leader β-cells immediately

disrupted pan-islet coordination, the use of “Flash-Seq” to photolabel and then perform single cell RNA Seq revealed that mouse leader β-cells have a discrete transcriptome *versus* follower cells (Chabosseau et al, 2022). Nevertheless, the mechanisms through which the coordination between leader and follower cells is achieved largely remain unclear. Here, we will discuss our optogenetic and optopharmaceutical approaches to dissecting the mechanisms through which leader β-cells initiate and spread calcium waves to follower cells. In particular, we seek to explore whether membrane depolarization and Ca^{2+} influx, and the mobilization of ER Ca^{2+} stores, play equivalent roles in the activation of leader cells and in Ca^{2+} wave propagation. To achieve plasma membrane depolarization, we will employ the blue optogenetic stimulatory channelrhodopsin CheRiff. Similarly, to explore the role of ER Ca^{2+} store mobilization, we will employ photocaged inositol 1,4,5 *tris*phosphate (IP_3) and RNAi-based or CRISPR-Cas9 inactivation of Sarco(endo)plasmic reticulum Ca^{2+}-ATPase-2 (SERCA2). Using both isolated islets, and islet engraftment into the anterior chamber of the mouse eye, these studies should provide further insights into the mechanisms through which β-cell heterogeneity enables the efficient stimulation of insulin secretion.

A Short-Term High-Fat, Low-Carbohydrate Diet Mitigates Glucose Intolerance and Improves Insulin Secretion in Mice Carrying the HNF-1αG319S Variant

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Background: In Manitoba, 40% of Indigenous youth with type 2 diabetes (T2D) carry a variant in the HNF-1a gene (HNF-1aG319S). The G319S variant is thought to drive pancreatic β-cell dysfunction; however, youth-onset T2D is a relatively recent phenomenon. We hypothesize the G319S variant impairs insulin secretion when exposed to dietary carbohydrate stress but is protective in the context of traditional off-the-land foods rich in fat and protein.

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

Results: A HFHC diet accelerated the development of glucose intolerance and impaired GSIS in G319S-expressing female mice between 12- and 24-weeks-of-age. Conversely, consuming a HFLC diet improved glucose tolerance in males and prevented GSIS impairments in females at 12-weeks-of-age. However, a long-term HFLC diet induced glucose intolerance and insulin hypersecretion across all genotypes and sexes.

Conclusion: The short-term consumption of a HFLC diet normalizes insulin secretion and glucose tolerance in G319S carriers, although long-term intake is less favourable. Conversely, a present-day HFHC diet accelerates metabolic dysfunction and impairs insulin secretion in G319S-expressing mice. These studies may inform nutritional interventions for youth with T2D while ultimately supporting community-led efforts to access off-the-land traditional foods.

Ketogenic Diet Intervention Increases *In Vivo* Incretin Response but Not GSIS

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Type 2 Diabetes (T2D) is a progressive, metabolic disease involving impaired islet function and insulin sensitivity. Diet intervention, recently with the ketogenic diet (KD), is often recommended to manage glycemia. The KD is an extremely high fat, low carbohydrate diet that can induce a fasting-like state, increasing the production and utilization of ketone bodies. The KD has had favorable glycemic and weight management outcomes in short-term human studies in which compliance is maintained, but preclinical studies have not yet investigated the role of long-term KD on islet function. To induce metabolic dysfunction, we fed mice a high fat diet (HFD) for 22 weeks or 10 weeks followed by 12 weeks intervention of a KD or their baseline grain-based diet (GBD). KD-fed mice had elevated fed ketones confirming a state of nutritional ketosis. Additionally, KD-fed female mice had significantly lower weight over time than HFD continuers. After glucose gavage, plasma glucose was not different between groups; but active incretins were elevated in KD mice. This did not result in increased glucose-stimulated insulin secretion in response. At sacrifice, weight and liver lipids were reduced in KD intervention mice; however, glucose stimulated insulin secretion (GSIS) in isolated islets was not rescued by intervention. Together, KD intervention may have modest weight benefit and increase incretin response but does not improve GSIS in obese male or female mice.

***Cyp1a1/1a2* Deletion Protects Female Mice from High-Fat Diet-Induced Glucose Intolerance**

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Epidemiological data suggest that external stressors, such as a high-fat diet (HFD) and exposure to environmental pollutants, increase Type 2 diabetes (T2D) risk. Cytochrome P450 1A1/1A2 (CYP1A1/1A2) are enzymes primarily involved in xenobiotic metabolism, but they are also involved in other processes, such as fatty acid metabolism. Notably, CYP1A1/1A2 are upregulated in mouse islets following HFD-feeding. We hypothesized that chronic activation of CYP1A1/1A2 is detrimental to β-cell health and function as CYP1A1/1A2 activity can produce harmful reactive oxygen species. Our goal is to determine whether CYP1A1/1A2 enzyme activity is involved in maintaining β-cell function under metabolic stress. We performed a long-term HFD study on male and female *Cyp1a1/1a2* global knockout (*Cyp*KO) mice (n=25) and wildtype (WT) littermate controls (n=29). Starting at ~31-weeks of age, half of the mice were transferred to a 45% HFD and the other half remained on chow for 13 weeks; metabolic assessments were conducted throughout the study. *Cyp*KO females were partially protected from HFD-induced glucose intolerance compared to WT females, but they exhibited similar levels of insulin resistance. *Cyp*KO females had lower plasma insulin levels *in vivo* and suppressed insulin secretion by isolated islets *ex vivo* compared to WT females. There were no genotype-based differences in glucose tolerance, insulin secretion, and insulin resistance in male mice on either diet. Collectively, our data suggest that CYP1A1/1A2 enzymes mediate glucose homeostasis and insulin secretion in a sex-specific manner.

Single-cell RNA Sequencing Reveals a Role for Reactive Oxygen Species and Peroxiredoxins in Fatty Acid-induced Rat ß-cell Proliferation

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Nutrients play an important role in β-cell compensation and dysfunction in obesity and Type 2 diabetes, respectively. Previously we demonstrated that fatty acids potentiate glucose-induced β-cell proliferation in response to nutrient surfeit in rats. In recent studies we identified the mono-unsaturated fatty acid oleate as a major driver of β-cell proliferation in rat islets ex vivo.

Aim: To identify mechanisms underlying fatty acid-induced β-cell proliferation using single-cell transcriptomic profiling.

Methods: Isolated rat islets were exposed to 16.7mM glucose +/- oleate or palmitate (0.5mM) for 48h. Proliferation was assessed by flow cytometry using C-peptide antibody and EdU. Single-cell cDNA libraries were generated using 10X Genomics technology and sequenced on the Illumina platform. Bioinformatic analyses were performed using the Cell Ranger pipeline and Seurat package.

Results: Oleate increased β-cell proliferation by 3.1+/-0.4 fold ($p< 0.01$; n=4). Bioinformatic analyses of single-cell RNA sequencing data revealed 5 β-cell sub-populations, including proliferating β-cells. Gene set enrichment analysis between proliferative and non-proliferative β-cells identified an increase in mitochondrial activity and reactive oxygen species-related pathways in proliferating β-cells, as well as an up-regulation of peroxiredoxins (Prdx1, Prdx2, Prdx4). Oleate-induced β-cell proliferation was reduced in the presence of the antioxidant N-acetylcysteine (0.73+/-0.14-fold, $p< 0.01$; n=5) or the peroxiredoxin inhibitor Conoidin-A (0.54+/-0.08 fold, $p< 0.01$; n=4).

Conclusion: Our data suggest that ROS production and peroxiredoxin signaling are required for oleate-induced β-cell proliferation in rat islets.

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

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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: Glycine currents were measured using the whole-cell patch-clamp technique in human β cells from donors with or without T2D, or after culture in 5.5 mmol/L or 15 mmol/L glucose for 2 days. The expression of the GlyR α1, α3, and β subunits mRNA splice variants was compared between islets from donors with and without T2D.

Results: The glycine-evoked currents in β cells from donors with T2D (-0.7690 pA/pF ± 0.5298, n=7) were smaller than those measured in cells from donors without diabetes (-9.679 pA/pF ± 2.068, n=29. The β cells cultured in 15 mmol/L glucose for 2 days had smaller glycine-evoked currents (-6.121 pA/pF ± 2.055, n=10) than those in control media (-13.16 pA/pF ± 3.746, n =11). However, the expression of most GlyR subunit mRNA splice variants was overall decreased in islets of donors with T2D, with no evidence of a shift in alternative splicing.

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.

The Impact of Inhibiting Prohormone Convertases on Human Pro-Islet Amyloid Polypeptide

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The β-cell prohormones, proinsulin and pro-islet amyloid polypeptide (proIAPP), are processed to their mature biologically active forms, insulin and IAPP, in the β-cell secretory pathway. This process involves the prohormone convertases PC1/3 and PC2. Individuals living with type 1 and type 2 diabetes exhibit persistent secretion of proinsulin and proIAPP forms, indicating impaired processing of β-cell prohormones. The current understanding of the mechanism of β-cell prohormone processing has mainly been derived from rodent studies. These studies have shown that both Pc1/3 and Pc2 mediate proinsulin and proIAPP processing in mice; however, recent data suggest that PC1/3 is essential for proinsulin cleavage in human β-cells and that PC2 plays little to no role. Our studies in mice have demonstrated that while Pc1/3 is sufficient for complete proinsulin processing, it is more efficient with Pc2 present, and that Pc2 is necessary for proIAPP processing in mice. We hypothesize that as in mice, PC2 plays an essential role in processing proIAPP in human β-cells, and inhibition of PC2 will result in accumulation of the intermediate, $proIAPP_{1-48}$. To elucidate the role of PC2 in human β-cell prohormone processing, here we propose to manipulate PC2 activity in primary human β-cells using enzyme inhibitors or CRISPR-based genome editing. The levels of $proIAPP_{1-67}$, $proIAPP_{1-48}$, and mature IAPP will be detected by western blot and enzyme-linked immunosorbent assay (ELISA). This study may identify new targets for therapeutics for type 1 and type 2 diabetes.

Role of the Interaction Between NCK1 and PERK in Pancreatic β-Cell Function and Survival

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Preventing pancreatic β-cell failure and death could help treat diabetes. Protein Kinase R–Like Endoplasmic Reticulum Kinase **(**PERK)**,** a player in the unfolded protein response, is essential for β-cell function and survival. PERK activity is modulated by binding of the adaptor protein NCK1 (Non-Catalytic region of tyrosine Kinase). Silencing of *Nck1* in β-cells *in vitro* enhances basal PERK activity and prevents its pathological hyperactivation under diabetogenic stresses,

improving β-cell function and survival. Therefore, we hypothesized that knockdown NCK1 *in vivo* could prevent β-cell failure and death in diabetes.

To this end, we generated mice with a β cell-specific knock-out of *Nck1* (NCK1-βKO mice). Glucose homeostasis was assessed and pancreatic insulin content and secretion measured in KOs and age- and sex-matched littermate controls. PERK signaling and β-cell survival was assessed in primary islets.

Healthy male and female NCK1-βKO mice had oral glucose and insulin sensitivity similar to control mice, despite increased total pancreatic insulin content in male NCK1-βKO mice*. In vitro*, insulin content of female NCK1-βKO islets tended to be improved in glucolipotoxic conditions. Glucose stimulated insulin secretion of male NCK1-βKO islets was lower in basal and glucolipotoxic conditions compared to control islets. In male NCK1-βKO islets, basal PERK pathway was increased compared to control. PERK pathway hyperactivation was prevented in male and female NCK1-βKO islets under stress conditions, which was associated with reduced cellular stress and death markers compared to controls.

This study suggests that silencing *Nck1* in mouse pancreatic β-cells could improve β-cell survival in response to diabetic stress.

Individual Variability Dicatates Cross-talk Between AhR and HIFα Signalling Pathways in Human Donor

NOA GANG, CAMERON D. G. SINCLAIR, WILLIAM G. WILLMORE, FRANCIS C. LYNN, and JENNIFER E. BRUIN

Man-made pollutants, such as dioxin-like and polyaromatic hydrocarbon-like compounds, are widely distributed in the environment, resulting in variable levels of exposure. Our lab has shown that 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD) activates the aryl hydrocarbon receptor (AhR) and upregulates the xenobiotic metabolism enzyme cytochrome P450 1A1 (CYP1A1) in pancreatic islets. The AhR pathway requires aryl hydrocarbon nuclear translocator (ARNT) as a binding partner to upregulate downstream gene targets. Hypoxia inducible factors (HIFs) also depend upon ARNT binding. In hepatocytes, simultaneous activation of AhR and HIF1α resulted in preferential upregulation of HIF1α target genes and CYP1A1 suppression. To investigate whether cross-talk between AhR and HIF1α occurs in human pancreatic endocrine cells, we exposed stem cell derived β-like (SC-β) cells and human donor islets to: a) vehicle + normoxia, b) TCDD (10 mM) + normoxia, c) vehicle + hypoxia (1% O_2), or d) TCDD + hypoxia for 48 hours. We measured expression of AhR and HIF1α gene targets to determine preferential pathway activation. In SC-β cells, hypoxia exposure consistently interfered with AhR activation by TCDD, but TCDD exposure did not interfere with HIF1α activation. In human islets, molecular cross-talk was variable between donors; hypoxia exposure interfered with AhR activation in only 2 of 5 donors. We speculate that this variability is related to previous environmental exposure, as it was not seen in environmentally naïve SC-β cells.

Session 4: It's not all about the β-cell, you know!

FFA4 Regulates Insulin Secretion via Inhibition of Somatostatin Secretion from Delta Cells

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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 target for type 2 diabetes therapy. FFA4 is expressed in islets, but its precise mechanism of action remains unknown. Previous studies from our group suggest that FFA4 regulates somatostatin secretion by delta cells.

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 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; $p<0.0001$). In isolated human islets, insulin secretion tended to increase from 4.8±0.6 to 5.8±1 % of insulin content in response to 10 μM of CpdA (n=3), while SST secretion decreased from 87.2±18.5 to 79.3±18.6 pM (n=3). No effect of CpdA on insulin or somatostatin secretion was observed in islets from somatostatin knockout mice (insulin: 3.2±0.01 vs 2.9±0.4, n=6, NS; somatostatin: 11.4±1.2 vs 11.9±1.2, n=6, NS). Likewise, preliminary data in islets isolated from diphtheria toxin-treated mice expressing the diphtheria toxin receptor under the somatostatin promoter showed no effect of CpdA on insulin or somatostatin release.

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

Master Gene Regulator Med15 is Required for Glucagon Expression in Adult Mouse Pancreatic Alpha Cells

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Pancreatic alpha (α-)cells secrete the hormone, glucagon, to trigger hepatic glucose production during hypoglycemia. This process is disrupted in diabetes, however, the transcriptional mechanisms behind this disruption are poorly understood. Our preliminary data demonstrated that transcriptional coregulator Mediator subunit 15 (Med15) is highly expressed in adult mouse and human α-cells; and mice which lack Med15 have fewer α-cells. We also showed that Med15 is enriched near genes that are essential for mouse α-cell function. These findings suggest that Med15 may regulate the expression of genes important for α-cell specification and function. To determine whether Med15 controls the maintenance of functional α-cells, we genetically deleted Med15 in mouse α-cells using a glucagon-expressing cell-specific Cre driver (Glucagon-iCre; αM15KO) and included a fluorescence Cre reporter ($Rosa26^{mTmG}$ Cre reporter) to isolate Cre-expressing cells. We showed that Cre-expressing pancreatic cells from 8-week-old αM15KO express lower levels of glucagon compared to those from littermate controls at the transcript and protein levels using qPCR and immunofluorescence respectively. These data suggest that Med15 is required for glucagon expression in α-cells. We will perform and integrate transcriptomic and Med15 DNA binding analyses on αM15KO and littermate controls to identify Med15-dependent transcriptional mechanisms that drive α-cell maintenance and function.

Exploring a Paradigm Shift in Islet Biology: The Emerging Role of α-cell Glucagon-Like Peptide-1 (GLP-1)

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Background: Glucagon-Like Peptide-1 (GLP-1) is released from intestinal endocrine cells in response to dietary glucose. GLP-1 enhances insulin production and secretion from pancreatic β-cells, and supports β-cell health during metabolic stress. In certain conditions, GLP-1 is produced by pancreatic alpha-cells. Alpha-cell GLP-1 acts on neighboring β-cells to improve their function and survival. We aim to identify the molecular mechanism that promotes GLP-1 production in the pancreas so that we may explore this pathway in future drug discovery.

Methods: Alpha-TC1/6 cells are cultured in control conditions (5.5mM glucose + saline vehicle) and proinflammatory conditions (interleukin-6 [IL-6], stromal-derived factor-1 [SDF-1]) for 24-72 hours. We harvest cells and assess changes proglucagon processing gene transcription and protein expression. Similar assays are conducted on FACS sorted human alpha cells from cadaveric organ donors distributed through IsletCore.

Results: While 72 hours in hyperglycemia elicits a modest 2-fold increase in *PCSK1* (prohormone convertase 1/3 gene) expression, 72hrs in IL-6 or SDF-1 cause a 16-, and 22- fold increase in gene expression, respectively. Both conditions significantly alter the proglucagon processing environment and enhance GLP-1 production.

Conclusions: Proinflammatory stimulation by IL-6 and SDF-1 are strong potentiators of *PCSK1* and PC1/3 expression in alpha cells. These conditions promote GLP-1 production, although the exact cell-signalling mechanism has yet to be determined.

The Diabetes-Related Gly482Ser Polymorphism Affects PGC1A Stability and Glucose Metabolism

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Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1A) 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 β-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 PGC1A 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.

Cisplatin Impairs Mitochondrial Function and Insulin Secretion in Mouse Islets

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Type 2 diabetes incidence is rising at an alarming rate across the globe. Previous research has shown increased rates of metabolic syndrome and Type 2 diabetes in cancer survivors following treatment with cisplatin, a commonly used chemotherapeutic agent. The goal of our research is to determine if cisplatin treatment puts cancer survivors at greater risk for developing Type 2 diabetes, due in part to off-target damage to pancreatic β-cells. To study the effects of cisplatin on β-cells, we treated immortalized β-cells (INS-1) and primary mouse islets with 10 μM cisplatin or vehicle for 48-hours *in vitro*. We found that INS-1 cells were more sensitive to cisplatin-induced cell death compared to a human colon carcinoma cell line. Cisplatin-exposed INS-1 cells also showed dysregulated glucose-stimulated insulin secretion in a static assay. We next confirmed these findings in primary mouse islets using a dynamic perifusion assay. Cisplatin-exposed islets displayed increased basal insulin secretion, impaired first-phase glucose-stimulated insulin secretion, and disrupted potassium chloride-induced insulin secretion, but normal insulin content. Cisplatin also profoundly decreased basal oxygen consumption rate and impaired ATP-linked respiration in islets, as measured by a Seahorse Analyzer. However, cisplatin-exposed islets had a greater spare respiratory capacity, a potential compensation mechanism to combat cellular stress. Our data thus far suggest that cisplatin treatment acutely disrupts rodent β-cell function. These off-target effects may contribute to increasing risk of developing Type 2 diabetes in cancer survivors following chemotherapy.

Control of Post-prandial Intestinal Metabolism by Gut Hormone Receptor Signalling

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Glucagon-like peptide 1 (GLP-1) and GLP-2 are gut-derived hormones secreted in response to a meal. GLP-1 receptor (GLP-1R) signalling promotes meal-stimulated insulin secretion, while GLP-2R signalling promotes intestinal growth; their roles in post-prandial intestinal metabolism remain unclear. Male and female mice were fasted for 24 hours – mice were euthanized in the fasted state or after 4 hours of refeeding. Compared to fasting, refeeding with a standard chow diet significantly reduced jejunal *Glp1r* mRNA expression in both male and female mice, however, *Glp2r* mRNA expression remained unchanged. In male wild-type mice, Nanostring mRNA analyses revealed that refeeding significantly upregulated jejunal mitochondrial respiration, amino acid synthesis, autophagy, and AMPK signalling pathways. In male *Glp1r*$^{-/-}$*Glp2r*$^{-/-}$ (GLPDRKO) mice, however, refeeding significantly downregulated all four pathways, suggesting that GLP-1R and/or GLP-2R signalling influence these pathways. In female wild-type mice, refeeding

significantly upregulated mitochondrial respiration and amino acid synthesis, however, in contrast to wild-type male mice, refeeding downregulated autophagy and AMPK signalling. In female GLPDRKO mice, refeeding also downregulated autophagy and AMPK signalling, however, refeeding downregulated both mitochondrial respiration, amino acid synthesis, suggesting that GLP-1R and/or GLP-2R control the latter two pathways, independent of sex. Overall, these data reveal dynamic changes in jejunal gene expression changes regulated by nutrient availability and local signalling circuits engaged by GLP-1R and/or GLP-2R signalling. Identifying the intestinal GLP-1R and GLP-2R-expressing cells responsible for this metabolic switch will help elucidate the intestinal changes induced during metabolic disease progression.

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

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Background: Genetic testing in Anishiniinew communities in central Canada led to the discovery of the HNF-1aG319S variant, which may contribute to youth-onset type 2 diabetes. HNF-1a is a transcription factor that controls glucose and lipid metabolism in the liver, and maintenance of pancreatic β-cell identity and function. Currently, it is unclear how the G319S variant influences these pathways. Given the metabolic demand associated with traditional lifestyle practices in central Canada, the G319S variant may instead confer an advantage to prolonged fasting. Here, we examine the impact of prolonged fasting in G319S expressing mice compared to control mice fed a standard chow diet.

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

Results: A statistically significant reduction in liver triglycerides was observed in G/S ($p=0.0237$) and S/S ($p=0.0185$) mice compared to G/G. In addition, increased expression of genes involved in cholesterol synthesis and ketogenesis was observed, including HMGCR in G/S ($p=0.0140$) and S/S ($P=0.0073$) mice, as well as increased expression of genes involved in gluconeogenesis, including G6PT-1 in S/S mice ($p=0.0290$). Once fasted, a decrease in blood glucose was observed in G/S ($P=<0.0001$), and S/S ($P=0.0385$) mice compared to G/G, and a trend towards increased blood ketones was also seen. In pancreatic islets, a reduction in insulin content was seen in G/S ($p=0.0175$) and S/S ($p=0.0065$) mice compared to G/G. EM images showed an increase percentage of immature insulin granules in male S/S ($p=0.0157$) compared to G/G, and increased percentage of rod-shaped insulin granules in G/S mice compared to S/S.

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

Discovering Novel Therapeutic Compounds for Type 1 Diabetes by Depleting Autoreactive T Cells

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Background: Type 1 diabetes (T1D) accounts for about 10% of all diagnosed cases of diabetes. Patients with T1D require lifelong insulin injections due to profound loss of insulin-producing β-cells. Autoreactive T cells are the main cell type responsible for promoting β-cell death; hence, depletion of autoreactive T cells could have a protective effect on β-cells. Our lab has demonstrated that the interactions between the scaffold protein 14-3-3zeta and BCL-2 family proteins, such as BAD, can prevent cell apoptosis. Thus, we hypothesize that disrupting 14-3-3zeta:BAD interactions could be harnessed as a sensor to discover pro-apoptotic compounds for autoreactive T cells to treat T1D.

Method: We developed bioluminescence resonance energy transfer (BRET) sensors by fusing luciferase to 14-3-3zeta and mCitrine to BAD fragments, respectively. The capacity of the sensor to detect 14-3-3zeta:BAD interactions was validated with two 14-3-3 inhibitors (FTY720 and 14-3-3i). The sensors were used to screen for hits that can disrupt 14-3-3zeta:BAD interactions from an FDA-approved drug library in a high-throughput format. A robotic liquid handling system was used to aid the primary screening.

Results: 154 drugs were identified. These drugs are tested on CD8 T cell lines and primary GFP-expressing CD8+ T cells from our transgenic non-obese diabetic mice to confirm their capacity to specifically induce T cell apoptosis.

Conclusion: Our research may lead to the discovery of novel small molecule drugs that protect β-cell from immune attach and delay the progression of T1D.