2016 RESEARCH DAY

08.30–17.00 | Lister Hall, Maple Leaf Room | University of Alberta

TUESDAY OCTOBER 4

KEYNOTE SPEAKER

SHEILA COLLINS, PhD
Professor, Integrative Metabolism Program
Sanford Burnham Prebys Medical Discovery Institute
2016 ADI RESEARCH DAY
Tuesday October 4
LISTER HALL, Maple Leaf Room, University of Alberta

CONTENTS

1. Note From the Director Page i
2. Program Summary Pages ii-iii
3. Sheila Collins, PhD Page iv
4. Prizes Page v
5. Abstracts - Table of Contents Pages A-F
6. Oral Presentation Abstracts Pages 1-20
7. Poster Presentation Abstracts Pages 21-57

Thank you to our generous event sponsors MERCK and Eli Lilly and to the Alberta Diabetes Foundation for providing door prizes.

Leading the World in the Prevention, Treatment and Cure of Diabetes
Welcome to the 2016 ADI Research Day. Activities at the ADI range from the study of immunology, basic cell/molecular biology and bioengineering to clinical research, nutrition and exercise, population health and the development of public policy in diabetes care. We also continue to grow – we now have over 60 investigators and 200 trainees/staff making us one of the largest and most active diabetes centres worldwide. A testament to this is the new location for today – we have already outgrown the ADI in just a few short years!

This annual event is intended to provide a forum to showcase the research efforts of our ADI trainees – this year our trainees will present 12 full oral presentations, 8 mini oral presentations, and 37 poster presentations. For some of our trainees it will be the first time presenting their research in front of an audience of their peers and senior scientists. Today is about giving you (the next generation of diabetes researchers!) an opportunity to present your most recent exciting results and ideas.

Research at the Alberta Diabetes Institute is made possible by your dedication and excellence. Through your efforts, we are ideally positioned to continue to make major advances in the prevention and treatment of diabetes, and ultimately to find a cure. We hope that you will be inspired by your peers to continue to excel in your scientific endeavors and I encourage you to ask questions during both the talks and poster sessions.

Thank you to the Alberta Diabetes Foundation for their continued efforts in raising funds to support our research projects and trainees. And finally, I would also like to take this opportunity to thank our sponsors and Merck Canada and Eli Lilly Canada for their generous support of this research day and for sponsoring the visit of our esteemed guest speaker, Dr. Sheila Collins.

Best Regards,

Peter Light, PhD
Director, Alberta Diabetes Institute
Dr. Charles A. Allard Chair in Diabetes Research
Professor of Pharmacology
2016 ADI RESEARCH DAY
Tuesday October 4
LISTER HALL, Maple Leaf Room, University of Alberta

MORNING SESSION

0830-0845  WELCOME AND OVERVIEW  Dr Peter Light, Director, Alberta Diabetes Institute

Keynote Speaker

0845-0945  Dr Sheila Collins  Sanford Burnham Prebys Medical Discovery Institute, Florida, USA
Introduction: Dr Peter Light

Evolving role of adipose tissue: from storage locker to metabolic integrator

SESSION 1

Chair - Jeffrey Johnson

0945-1000  Anne-Francoise CLOSE  Supervisor: Buteau
THE NUCLEAR RECEPTOR NOR1/NR4A3, INDUCES BETA-CELL APOPTOSIS BY TARGETING THE MITOCHONDRIA

1000-1015  Ibrahim ADAM  Supervisor: West
THE IMPORTANCE OF CD4+ T CELLS IN ANTIBODY RESPONSE TO BLOOD GROUP ANTIGENS

1015-1030  Keshav GOPAL  Supervisor: Ussher
FOXO1-MEDIATED REGULATION OF PYRUVATE DEHYDROGENASE AND GLUCOSE OXIDATION IN DIABETIC CARDIOMYOPATHY

1030-1038  Gayathri ANANTHAKRISHNAN  Supervisor: Vine
Mini talk: ANDROGENS MODULATE LIPID METABOLISM AND LIPIDOGENIC PATHWAYS IN A RODENT MODEL OF METABOLIC SYNDROME AND POLYCYSTIC OVARY SYNDROME

1038-1045  Thea LUIG  Supervisor: Campbell-Scherer
Mini talk: STRATEGIES FOR COMPLEX INTERVENTION IMPLEMENTATION IN PRIMARY CARE: THE INTERACTIVE PROCESS FRAMEWORK AND THE 5AS TEAM OBESITY STUDY

1045-1100  BREAK  Glacier Room

SESSION 2

Chair - Cathy Chan

1100-1115  Yang YU  Supervisor: Ungrin
CHARACTERIZING AND OPTIMIZING PSEUDOISLETS

1115-1130  Victoria BARONAS  Supervisor: Kurata
Kv1.2 CHANNELS AT THE INTERFACE OF REDOX AND ELECTRICALexcitability

1130-1145  Andrew PEPPER  Supervisor: Shapiro
TRANSPLANTATION OF HUMAN DERIVED PANCREATIC ENDODERM CELLS REVERSED DIABETES POST-TRANSPLANTATION INTO A PREVASCULARIZED SUBCUTANEOUS SITE

1145-1152  Trevor POITRAS  Supervisor: Zochodne
Mini talk: MAJOR URINARY PROTEIN: UNEXPECTED EXPRESSION AND ROLES IN DIABETIC PERIPHERAL NEURONS

1153-1200  Maxim ESKIN  Supervisor: Eurich
Mini talk: IMPACT OF DRUG EXPOSURE DEFINITIONS ON OBSERVED ASSOCIATION IN PHARMACOEPIDEMIOLOGY RESEARCH

1200-1230  LUNCH  Glacier Room

1230-1400  POSTER SESSION  MAPLE LEAF ROOM
# 2016 ADI Research Day

Tuesday October 4  
Lister Hall, Maple Leaf Room, University of Alberta

## Afternoon Session

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Chair</th>
<th>Title</th>
<th>Presenter</th>
<th>Supervisor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1230-1400</td>
<td>Poster Presentations</td>
<td></td>
<td>Posters located at the back of Maple Leaf Room</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1415-1430</td>
<td>Session 3</td>
<td>Jane Yardley</td>
<td>REGULATORS OF GLYCINE SIGNALING IN HUMAN PANCREATIC beta cells</td>
<td>Richard Yando</td>
<td>MacDonald</td>
</tr>
<tr>
<td>1430-1445</td>
<td></td>
<td></td>
<td>ADROPIN ENHANCEMENT OF CARDIAC INSULIN SENSITIVITY AND INHIBITION OF FATTY ACID OXIDATION ARE ASSOCIATED WITH IMPROVEMENT OF CARDIAC FUNCTION AND EFFICIENCY</td>
<td>Tariq Altamimi</td>
<td>Lopaschuk</td>
</tr>
<tr>
<td>1445-1500</td>
<td></td>
<td></td>
<td>PREVALENCE AND INCIDENCE OF DIABETES IN ALBERTA'S TOMORROW PROJECT COHORT - LINKAGE WITH ADMINISTRATIVE HEALTHCARE DATA</td>
<td>Ming YE</td>
<td>Johnson J</td>
</tr>
<tr>
<td>1500-1507</td>
<td></td>
<td></td>
<td>Mini talk: A MINIMUM CONDITIONING PROTOCOL TOWARDS TRANSPLANTATION TOLERANCE IN NOD MICE BY MIXED HEMATOPOIETIC CHIMERISM</td>
<td>Jiaxin Lin</td>
<td>Anderson</td>
</tr>
<tr>
<td>1508-1515</td>
<td></td>
<td></td>
<td>Mini talk: ALTERED DIETARY LIPID METABOLISM IN A NOVEL MOUSE MODEL WITH INTESTINAL-SPECIFIC DELETION OF CTα</td>
<td>John Kennelly</td>
<td>Jacobs</td>
</tr>
<tr>
<td>1515-1530</td>
<td></td>
<td></td>
<td>Glacier Room</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1530-1545</td>
<td>Session 4</td>
<td>Lori West</td>
<td>IMPROVED GLUCOSE HOMEOSTASIS IN OBESE MICE IS ASSOCIATED WITH RESVERATROL-MEDIATED ALTERATIONS IN THE GUT MICROBIOME</td>
<td>Ty Kim</td>
<td>Dyck</td>
</tr>
<tr>
<td>1545-1600</td>
<td></td>
<td></td>
<td>PURE PRAIRIE LIVING PROGRAM - COMMUNITY BASED LIFESTYLE INTERVENTION</td>
<td>Fatheema Subhan</td>
<td>Chan</td>
</tr>
<tr>
<td>1600-1615</td>
<td></td>
<td></td>
<td>EXAMINING THE UTILITY OF BMX-001, A NOVEL REDOX-ACTIVE METALLOPORPHYRIN, IN A MURINE SYNGENEIC, MARGINAL ISLET TRANSPLANT MODEL</td>
<td>Antonio Brunini</td>
<td>Shapiro</td>
</tr>
<tr>
<td>1615-1622</td>
<td></td>
<td></td>
<td>Mini talk: PEMT-DEFICIENCY IMPROVES HEPATIC INSULIN SIGNALING</td>
<td>Jelske Van Der Veen</td>
<td>Vance</td>
</tr>
<tr>
<td>1623-1630</td>
<td></td>
<td></td>
<td>Mini talk: ACTIVATION OF PKD1 BY AUTOCRINE ATP SIGNALING IN PANCREATIC β CELLS</td>
<td>Shara Khan</td>
<td>MacDonald</td>
</tr>
<tr>
<td>1650-1715</td>
<td></td>
<td></td>
<td>WRAP UP AND PRIZES</td>
<td>Dr Peter Light</td>
<td></td>
</tr>
</tbody>
</table>
2016 ADI RESEARCH DAY
Tuesday October 4
LISTER HALL, Maple Leaf Room, University of Alberta

KEYNOTE SPEAKER

Sheila Collins, PhD

Dr. Collins received her Bachelor’s degree with Honors in Zoology from the University of Massachusetts at Amherst and then worked as a research technician at the Massachusetts General Hospital (Boston, MA) and the California Institute of Technology (Pasadena, CA) in developmental and molecular biology. Dr. Collins received her Doctorate in biochemistry and drug metabolism from the Massachusetts Institute of Technology with Michael Marletta, and conducted postdoctoral research with Robert Lefkowitz at Duke University, both of whom are members of the National Academy of Sciences. Dr. Collins continued her research career at Duke University Medical Center as a faculty member in the Department of Psychiatry and Behavioral Sciences, where she was awarded tenure and later moved to The Hamner Institutes for Health Sciences, helping to develop biomedical research, while retaining her Duke Faculty appointment. In 2010, Dr. Collins joined the Center for Metabolic Origins of Disease at Sanford Burnham Prebys Medical Discovery Institute as Professor of Metabolic Signaling and Disease. Dr. Collins has served on numerous review committees and advisory panels for the National Institutes of Health, the American Diabetes Association, American Heart Association, and has been an organizer of many national and international scientific meetings. Dr. Collins investigates biochemical mechanisms regulating body weight. Specifically, her lab is deciphering how hormonal signals such as adrenaline and novel pathways using heart-derived hormones control fat cell metabolism, including how the genes in brown and white adipocytes are controlled to increase energy expenditure and weight loss.
Prize announcements at 5:00 pm

**ORAL PRESENTATION AWARDS**

**Mini Oral Presentation**
8 entries
- 1 Best Award
- 2 Honourable Mention Award

**Full Oral Presentation**
12 entries
- 1 Best Award
- 2 Honourable Mention Award

**POSTER PRESENTATION AWARDS**

**Junior Posters (Summer Students)**
16 entries
- 2 Best Award
- 2 Honourable Mention Award

**Junior Posters (MSc Students)**
5 entries
- 1 Best Award
- 1 Honourable Mention Award

**Senior Posters (PhD Students)**
9 entries
- 1 Best Award
- 1 Honourable Mention Award

**Senior Posters (Postdocs, Research Associates, Technicians)**
7 entries
- 1 Best Award
- 1 Honourable Mention Award

**TRAINEE CHOICE AWARD**
- 1 Trainee Choice Award (only trainees vote – fill in ballot, their choice of best oral presentation)

**DOOR PRIZES**
- Door prize name draws at end of ADI Research Day, must be in attendance to collect a prize!
## 2016 ADI RESEARCH DAY
**Tuesday October 4**
LISTER HALL, Maple Leaf Room, University of Alberta

### ABSTRACTS

**ORAL PRESENTATION**
*(in order of presentation)*

<table>
<thead>
<tr>
<th>Page</th>
<th>Presenting Author</th>
<th>Supervisor</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CLOSE Anne-Francoise</td>
<td>BUTEAU</td>
<td>THE NUCLEAR RECEPTOR NOR1/NR4A3, INDUCES BETA-CELL APOPTOSIS BY TARGETTING THE MITOCHONDRIA</td>
</tr>
<tr>
<td>2</td>
<td>ADAM Ibrahim</td>
<td>WEST</td>
<td>THE IMPORTANCE OF CD4+ T CELLS IN ANTIBODY RESPONSE TO BLOOD GROUP ANTIGENS</td>
</tr>
<tr>
<td>3</td>
<td>GOPAL Keshav</td>
<td>USSHER</td>
<td>FOXO1-MEDIATED REGULATION OF PYRUVATE DEHYDROGENASE AND GLUCOSE OXIDATION IN DIABETIC CARDIOMYOPATHY</td>
</tr>
<tr>
<td>4</td>
<td>ANANTHAKRISHNAN Gayathri</td>
<td>VINE</td>
<td>ANDROGENS MODULATE LIPID METABOLISM AND LIPIDOGENIC PATHWAYS IN A RODENT MODEL OF METABOLIC SYNDROME AND POLYCYSTIC OVARY SYNDROME</td>
</tr>
<tr>
<td>5</td>
<td>LUIG Thea</td>
<td>CAMPBELL-SCHERER</td>
<td>STRATEGIES FOR COMPLEX INTERVENTION IMPLEMENTATION IN PRIMARY CARE: THE INTERACTIVE PROCESS FRAMEWORK AND THE 5AS TEAM OBESITY STUDY</td>
</tr>
<tr>
<td>6</td>
<td>YU Yang</td>
<td>UNGRIN</td>
<td>CHARACTERIZING AND OPTIMIZING PSEUDOISLETS</td>
</tr>
<tr>
<td>7</td>
<td>BARONAS Victoria</td>
<td>KURATA</td>
<td>Kv1.2 CHANNELS AT THE INTERFACE OF REDOX AND ELECTRICAL EXCITABILITY</td>
</tr>
<tr>
<td>8</td>
<td>PEPPER Andrew</td>
<td>SHAPIRO</td>
<td>TRANSPLANTATION OF HUMAN DERIVED PANCREATIC ENDODERM CELLS REVERSED DIABETES POST-TRANSPLANTATION INTO A PREVASCULARIZED SUBCUTANEOUS SITE</td>
</tr>
<tr>
<td>9</td>
<td>POITRAS Trevor</td>
<td>ZOCHODNE</td>
<td>MAJOR URINARY PROTEIN: UNEXPECTED EXPRESSION AND ROLES IN DIABETIC PERIPHERAL NEURONS</td>
</tr>
<tr>
<td>10</td>
<td>ESKIN Maxim</td>
<td>EURICH</td>
<td>IMPACT OF DRUG EXPOSURE DEFINITIONS ON OBSERVED ASSOCIATION IN PHARMACOEPIDEMIOLOGY RESEARCH</td>
</tr>
<tr>
<td>11</td>
<td>YAN-DO Richard</td>
<td>MACDONALD</td>
<td>REGULATORS OF GLYCINE SIGNALING IN HUMAN PANCREATIC BETA CELLS</td>
</tr>
<tr>
<td>12</td>
<td>ALTAMIMI Tariq</td>
<td>LOPASCHUK</td>
<td>ADROPIN ENHANCEMENT OF CARDIAC INSULIN SENSITIVITY AND INHIBITION OF FATTY ACID OXIDATION ARE ASSOCIATED WITH IMPROVEMENT OF CARDIAC FUNCTION AND EFFICIENCY</td>
</tr>
<tr>
<td>13</td>
<td>YE Ming</td>
<td>JOHNSON Jeffrey</td>
<td>PREVALENCE AND INCIDENCE OF DIABETES IN ALBERTA'S TOMORROW PROJECT COHORT - LINKAGE WITH ADMINISTRATIVE HEALTHCARE DATA</td>
</tr>
<tr>
<td>14</td>
<td>LIN Jiaxin</td>
<td>ANDERSON</td>
<td>A MINIMUM CONDITIONING PROTOCOL TOWARDS TRANSPLANTATION TOLERANCE IN NOD MICE BY MIXED HEMATOPOIETIC CHIMERISM</td>
</tr>
<tr>
<td>Page</td>
<td>Presenting Author</td>
<td>Supervisor</td>
<td>Title</td>
</tr>
<tr>
<td>------</td>
<td>------------------</td>
<td>------------</td>
<td>-------</td>
</tr>
<tr>
<td>15</td>
<td>KENNELLY John</td>
<td>JACOBS</td>
<td>ALTERED DIETARY LIPID METABOLISM IN A NOVEL MOUSE MODEL WITH INTESTINAL-SPECIFIC DELETION OF CTα</td>
</tr>
<tr>
<td>16</td>
<td>KIM Ty</td>
<td>DYCK</td>
<td>IMPROVED GLUCOSE HOMEOSTASIS IN OBESE MICE IS ASSOCIATED WITH RESVERATROL-MEDIATED ALTERATIONS IN THE GUT MICROBIOME</td>
</tr>
<tr>
<td>17</td>
<td>SUBHAN Fatheema</td>
<td>CHAN</td>
<td>PURE PRAIRIE LIVING PROGRAM - COMMUNITY BASED LIFESTYLE INTERVENTION</td>
</tr>
<tr>
<td>18</td>
<td>BRUNI Antonio</td>
<td>SHAPIRO</td>
<td>EXAMINING THE UTILITY OF BMX-001, A NOVEL REDOX-ACTIVE METALLOPORPHYRIN, IN A MURINE SYNGENEIC, MARGINAL ISLET TRANSPLANT MODEL</td>
</tr>
<tr>
<td>19</td>
<td>VAN DER VEEN Jelske</td>
<td>VANCE</td>
<td>PEMT-DEFICIENCY IMPROVES HEPATIC INSULIN SIGNALING</td>
</tr>
<tr>
<td>20</td>
<td>KHAN Shara</td>
<td>MACDONALD</td>
<td>ACTIVATION OF PKD1 BY AUTOCRINE ATP SIGNALING IN PANCREATIC β CELLS</td>
</tr>
<tr>
<td>Page</td>
<td>Presenting Author</td>
<td>Supervisor</td>
<td>Title</td>
</tr>
<tr>
<td>------</td>
<td>-------------------</td>
<td>------------</td>
<td>-------</td>
</tr>
<tr>
<td>21</td>
<td>ALGHAMDI Abdulrhman</td>
<td>JOHNSON Steven / MATHE Nonsi</td>
<td>SELF-REPORTED PHYSICAL ACTIVITY AMONG WOMEN WITH A PREVIOUS GESTATIONAL DIABETES PREGNANCY</td>
</tr>
<tr>
<td>22</td>
<td>BOIVIN Eric</td>
<td>RAYAT</td>
<td>THE EFFECTS OF NATURALLY DERIVED FRUIT EXTRACTS ON THE VIABILITY OF NEONATAL PORCINE ISLETS</td>
</tr>
<tr>
<td>23</td>
<td>CRODEN Jennifer</td>
<td>RAYAT</td>
<td>TREATMENT OF HUMAN ISLETS WITH CYANIDIN-3-O-GLUCOSIDE LEADS TO AN AUGMENTATION OF AUTOPHAGIC FLUX AND A DECREASED INFLAMMATORY RESPONSE IN VITRO</td>
</tr>
<tr>
<td>24</td>
<td>FATEHI Mortaza</td>
<td>CHAN</td>
<td>REGULAR-FAT CHEESE IMPROVES INSULIN SENSITIVITY IN A PRE-DIABETIC RAT MODEL</td>
</tr>
<tr>
<td>25</td>
<td>FUNK Deanna</td>
<td>YARDLEY</td>
<td>ISLET TRANSPLANT PATIENTS DO NOT DISPLAY HYPOGLYCEMIA DURING OR AFTER MODERATE AEROBIC EXERCISE</td>
</tr>
<tr>
<td>26</td>
<td>HAJAR Ali</td>
<td>URSCHEL / WEST</td>
<td>AGE-RELATED DIFFERENCES IN THE REGULATORY CAPACITY OF CD5+CD1D+ B-CELLS IN THE CONTEXT OF HEART GRAFT ACCEPTANCE</td>
</tr>
<tr>
<td>27</td>
<td>HE Xiao Tian</td>
<td>HAQQ</td>
<td>SLEEP DISORDERED BREATHING IN CHILDREN WITH PRADER-WILLI SYNDROME</td>
</tr>
<tr>
<td>28</td>
<td>KIM Ryek</td>
<td>MACDONALD</td>
<td>SECRETORY CHANGE OF CRYOPRESERVED HUMAN ISLETS AFTER CULTURE UNDER MATURATION CONDITIONS</td>
</tr>
<tr>
<td>29</td>
<td>KIM Tiffany</td>
<td>URSCHEL / WEST</td>
<td>LYMPHOCYTE ALTERATIONS IN HEART-TRANSPLANTED CHILDREN IN RELATION TO DEVELOPMENT OF ALLERGIC AND AUTOIMMUNE DISORDERS</td>
</tr>
<tr>
<td>30</td>
<td>KONDRO Douglas</td>
<td>UNGRIN</td>
<td>BIOPRINTING HIGH CELL DENSITY AND VASCULARIZED TISSUES</td>
</tr>
<tr>
<td>31</td>
<td>PURICH Kieran</td>
<td>SHAPIRO</td>
<td>THE IMPACT OF VASCULAR ENDOTHELIAL GROWTH FACTOR ON ANGIOGENESIS IN A SUBCUTANEOUS ISLET TRANSPLANT SITE.</td>
</tr>
<tr>
<td>32</td>
<td>RAFIEI Yasmin</td>
<td>SHAPIRO</td>
<td>IN VITRO EVALUATION OF THE OPTIMAL IMMUNOSUPPRESSIVE RATIO OF TREGS TO ISLETS</td>
</tr>
<tr>
<td>33</td>
<td>RODRIGUES SILVA Josue</td>
<td>RAYAT</td>
<td>CYANIDIN-3-O-GLUCOSIDE (C3G) IMPROVES THE VIABILITY AND FUNCTION OF HUMAN ISLET CELLS TREATED WITH HUMAN AMYLIN OR Aβ1-42 IN VITRO</td>
</tr>
<tr>
<td>34</td>
<td>SOSNIUK Morgan</td>
<td>WEST</td>
<td>OLD DOGS WITH NEW TRICKS: OPTIMIZING MIXED LYMPHOCYTE REACTION WITH DURACLONE FLOW CYTOMETRY PHENOTYPING</td>
</tr>
<tr>
<td>35</td>
<td>WANG Yiqun</td>
<td>WEST</td>
<td>BLOOD GROUP A-ANTIGEN-SPECIFIC TOLERANCE FOLLOWING INFANT A-ANTIGEN EXPOSURE IN A MOUSE MODEL OF ABO-INCOMPATIBLE HEART TRANSPLANTATION (ABOi HTx)</td>
</tr>
<tr>
<td>36</td>
<td>WILSON Hillary</td>
<td>FIELD</td>
<td>PREGNANT WOMEN WITH DIABETES MELLITUS IMPROVE THEIR DIET BY REDUCING SUGAR INTAKE.</td>
</tr>
</tbody>
</table>
## ABSTRACTS

### JUNIOR POSTER PRESENTATION

**MSc Students**

<table>
<thead>
<tr>
<th>Page</th>
<th>Presenting Author</th>
<th>Supervisor</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>ADAME PEREZ</td>
<td>MAGER</td>
<td>CHARACTERISTICS OF VITAMIN D SUPPLEMENT USERS VS NON SUPPLEMENT USERS IN AN AMBULATORY POPULATION WITH DIABETES AND CHRONIC KIDNEY DISEASE (CKD).</td>
</tr>
<tr>
<td>38</td>
<td>HAN</td>
<td>SAUVE</td>
<td>MITOCHONDRIAL DYSFUNCTION PRECEDES DIABETIC RETINOPATHY</td>
</tr>
<tr>
<td>39</td>
<td>MACDONALD</td>
<td>MAGER / HAQQ</td>
<td>BODY COMPOSITION, HANDGRIP STRENGTH, PHYSICAL CAPACITY AND MARKERS OF CARDIOMETABOLIC AND LIVER DYSFUNCTION IN CHILDREN WITH NONALCOHOLIC FATTY LIVER DISEASE AND PRADER-WILLI SYNDROME</td>
</tr>
<tr>
<td>40</td>
<td>WAN</td>
<td>VANCE</td>
<td>INHIBITION OF PHOSPHATIDYLETHANOLAMINE N-METHYLTRANSFERASE WITH ANTISENSE OLIGONUCLEOTIDES</td>
</tr>
<tr>
<td>41</td>
<td>WANG</td>
<td>KURATA</td>
<td>STATE- AND USE-DEPENDENT BINDING OF KCNQ CHANNEL OPENERS</td>
</tr>
</tbody>
</table>
## ABSTRACTS

### SENIOR POSTER PRESENTATION

#### PhD Students

<table>
<thead>
<tr>
<th>Page</th>
<th>Presenting Author</th>
<th>Supervisor</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>ALDANA-HERNANDEZ Paulina</td>
<td>JACOBS</td>
<td>DOES DIETARY CHOLINE OR TMAO SUPPLEMENTATION INCREASE Atherosclerosis in \textit{Ldlr-/} MICE?</td>
</tr>
<tr>
<td>43</td>
<td>AVEDZI Hayford</td>
<td>JOHNSON Jeffrey</td>
<td>EXAMINING SEX DIFFERENCES IN GLYCEMIC INDEX KNOWLEDGE AND INTAKE AMONG INDIVIDUALS WITH TYPE 2 DIABETES</td>
</tr>
<tr>
<td>44</td>
<td>CAMPBELL Scott</td>
<td>LIGHT</td>
<td>IMPROVING ISLET SURVIVAL BY ELEVATION OF INTRA-ISLET GLP-1</td>
</tr>
<tr>
<td>45</td>
<td>FU Jianyang (Claude)</td>
<td>MACDONALD</td>
<td>CONTRIBUTION OF KV2.1 CLUSTERING TO INSULIN EXOCYTOSIS AND-beta-CELL DYSFUNCTION IN TYPE 2 DIABETES</td>
</tr>
<tr>
<td>46</td>
<td>HUANG Hui</td>
<td>CHAN</td>
<td>ENDOPLASMIC RETICULUM (ER) STRESS CHAPERONE PROTEINS AND ASSOCIATED INSULIN COMPENSATION IN NILE RATS: AN EMERGING MODEL FOR TYPE 2 DIABETES</td>
</tr>
<tr>
<td>47</td>
<td>HUANG Wenlong</td>
<td>RAYAT</td>
<td>PROLIFERATION RESPONSE OF PBMCs FROM HUMAN DONORS WITH OR WITHOUT TYPE 1 DIABETES MELLITUS AGAINST NEONATAL PORCINE ISLET CELLS \textit{IN VITRO}</td>
</tr>
<tr>
<td>48</td>
<td>KIM Robin</td>
<td>KURATA</td>
<td>RESCUE MECHANISMS FOR KATP LOSS OF FUNCTION MUTATIONS HIGHLIGHT ESSENTIAL RESIDES AT THE KIR6.2 CHANNEL DOMAIN INTERFACE</td>
</tr>
<tr>
<td>49</td>
<td>ONDRUSOVA Katarina</td>
<td>LIGHT</td>
<td>BLUE LIGHT (470 nm) INDUCES PHENOTYPIC AND FUNCTIONAL CHANGES IN ADIPOCYTES VIA A MELANOPSIS-TRPC CHANNEL SIGNALING PATHWAY</td>
</tr>
<tr>
<td>50</td>
<td>PINTO Camila</td>
<td>PRADO</td>
<td>CREATINE SUPPLEMENTATION IN COMBINATION WITH RESISTANCE TRAINING IMPROVES GLYCEMIC CONTROL IN THE ELDERLY</td>
</tr>
</tbody>
</table>
## ABSTRACTS

### SENIOR POSTER PRESENTATION

Postdocs, Research Associates, Technicians

<table>
<thead>
<tr>
<th>Page</th>
<th>Presenting Author</th>
<th>Supervisor</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>AL BATRAN Rami</td>
<td>USSHER</td>
<td>RANOLAZINE TREATMENT IMPROVES GLYCEMIA AND DECREASES BODY WEIGHT IN OBESE AND INSULIN RESISTANT MICE</td>
</tr>
<tr>
<td>52</td>
<td>DADHEECH Nidheesh</td>
<td>BUTEAU</td>
<td>THE TYROSINE PROTEIN KINASE LYN: A NOVEL REGULATOR OF BETA-CELL PROLIFERATION AND SURVIVAL</td>
</tr>
<tr>
<td>53</td>
<td>MATHE Nonsi</td>
<td>JOHNSON Steven</td>
<td>OBJECTIVELY MEASURED DAILY PHYSICAL ACTIVITY AND SEDENTARY TIME IN WOMEN WITH PREVIOUS GESTATIONAL DIABETES MELLITUS</td>
</tr>
<tr>
<td>54</td>
<td>PURWANA Indri</td>
<td>BUTEAU</td>
<td>RESTORING HEAT SHOCK FACTOR 1 ACTIVITY TO PREVENT BETA-CELL APOPTOSIS</td>
</tr>
<tr>
<td>55</td>
<td>TOMS Derek</td>
<td>UNGRIN</td>
<td>RAPID OPTIMIZATION AND SCALE-UP OF CELL-BASED THERAPIES FOR TYPE 1 DIABETES</td>
</tr>
<tr>
<td>56</td>
<td>WANG Xiaofeng</td>
<td>CHAN</td>
<td>TRANS-11 VACCENIC ACID IMPROVES INSULIN SECRETION VIA GPR40 IN RAT AND HUMAN MODELS OF TYPE 2 DIABETES</td>
</tr>
<tr>
<td>57</td>
<td>WANG Ying</td>
<td>BUTEAU</td>
<td>GLUCOSYLCERAMIDE SYNTHASE, A CRITICAL REGULATOR OF BETA-CELL SURVIVAL</td>
</tr>
</tbody>
</table>
THE NUCLEAR RECEPTOR NOR1/NR4A3, INDUCES BETA-CELL APOPTOSIS BY TARGETING THE MITOCHONDRIA

Anne-Françoise Close, Barbara Villela, and Jean Buteau

Dept. of Agricultural, Food and Nutritional Sciences, University of Alberta

Background: Type 2 diabetes is characterized by a progressive deterioration of functional beta-cell mass. The mechanisms implicate low grade inflammation, the production of pro-inflammatory cytokines, and the subsequent up-regulation of beta-cell apoptosis. Understanding the molecular mechanisms underlying the regulation of beta-cell mass could lead to the development of new therapeutic approaches.

Our preliminary study identified the nuclear receptor Nor1 as a novel regulator of beta-cell mass. Indeed, Nor1 was up-regulated by pro-inflammatory cytokines in both INS cells and human islets. Nor1 KO animals displayed increased beta-cell mass and glucose tolerance. Moreover, Nor1 gain and loss of function studies revealed a key role of Nor1 in cytokine-mediated beta-cell apoptosis. We herein sought to precisely identify the molecular mechanisms by which Nor1 could regulate beta-cell life and death.

Methods: Subcellular localization of Nor1 was investigated using a Nor1GFP construct and Mitotracker®. Cells were transfected with pCMV-Nor1Flag in order to conduct gain-of-function experiments. Gene expression was evaluated by RNA sequencing. Glucose oxidation was measured using 14C-D-Glucose. ATP production and mitochondrial membrane potential were determined using Molecular Probes ATP determination kit A22066 and Cayman JC-1 assay kit 10009172 respectively. Mitochondrial morphology was studied by Transmission Electron Microscopy.

Results: Surprisingly, we detected a rapid translocation of Nor1 to the mitochondria in beta-cells exposed to cytokines. In addition, our genomic study revealed that Nor1 down-regulated the expression of genes encoded by the mitochondrial genome. This prompted us to further investigate the role of Nor1 at the mitochondria. Nor1 reduced glucose oxidation and ATP production in beta-cells. Consistently, Nor1 also modulated mitochondrial membrane potential. Electron microscopy images revealed that Nor1 induced mitochondrial fractionation and increased mitophagy.

Conclusion: These results unveil a new mode of action for Nor1 in regulating mitochondrial homeostasis.

Keywords: Beta-cell mass, Apoptosis, Cytokines, Nor1, Mitochondria.
THE IMPORTANCE OF CD4+ T CELLS IN ANTIBODY RESPONSE TO BLOOD GROUP ANTIGENS

Ibrahim Adam1,4, Bruce Motyka2,4, KeSheng Tao2,4, Lori West1,2,3,4.

1Medical Microbiology & Immunology, University of Alberta, Edmonton, AB, Canada; 2Pediatrics, University of Alberta, Edmonton, AB, Canada; 3Surgery, University of Alberta, Edmonton, AB, Canada; 4Alberta Transplant Institute, University of Alberta, Edmonton, AB, Canada

Background: ABO-incompatible heart transplantation (ABOi HTx) is safe during infancy and allows increased access to donors. B-cell tolerance develops to donor A/B antigen(s) (Ag) following ABOi HTx, but mechanisms of tolerance are not well-defined. Using recently developed A-transgenic (A-Tg) mice (B6 background) expressing human A-Ag on vascular endothelium and erythrocytes (RBC), we investigated the role of CD4+ T-cells in anti-A antibody (Ab) production.

Methods: Wild-type C57BL/6 mice (WT) were injected i.p. x3, 1 week apart with human blood group A RBC (hu-A) with (n=3) and without (n=6) CD4-depleting mAb (GK1.5), or A-Tg RBC (n=12) and adjuvant. Anti-A Ab in serum was measured by hemagglutination and ELISA (both IgG and IgM). Four weeks later, A-Tg RBC-injected mice were injected i.p. with hu-A-RBC; anti-A was measured again. To study the effect of human RBC-antigens, human group O (hu-O) and A-Tg RBC were mixed, injected i.p. x3, 1 week apart (n=5), then anti-A IgM titer was measured.

Results: Injection of hu-A RBC induced abundant anti-A Ab production (median titer 1:512). Following CD4+ T cell depletion, hu-A RBC injection failed to elicit anti-A Ab (titer <1:4). Despite comparable A-Ag expression, A-Tg RBC did not induce anti-A Ab (median titer ≤ 1:2), however, injection of hu-A RBC 4 weeks after A-Tg RBC injection elicited abundant anti-A Ab (median titer 1:256). Co-injection of A-Tg and hu-O RBC did not induce anti-A Ab (titer ≤1:2).

Conclusions: Administration of A-Ag alone (A-Tg RBC) did not stimulate an anti-A Ab response. This cannot be interpreted as tolerance because subsequent administration of hu-A RBC elicited anti-A Ab. In contrast, hu-A RBC (A-Ag plus foreign glycoproteins/glycolipids) induced a strong anti-A Ab response that was T cell-dependent. The lack of an anti-A response following co-injection of A-Tg RBC and hu-O RBC is consistent with a requirement for a chemical linkage of foreign protein/lipid with A-antigen. Contrary to accepted understanding, this study indicates that A/B Ags alone do not stimulate B cell responses without CD4+ T cell participation.

Key words: Transplantation, B cell, ABO-blood group
FOXO1-MEDIATED REGULATION OF PYRUVATE DEHYDROGENASE AND GLUCOSE OXIDATION IN DIABETIC CARDIOMYOPATHY

Keshav Gopal, Rami Al Batran, Hanin Aburasayn, Amina Eshreif, Farah Eaton, John R. Ussher

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta.

Background: Cardiovascular disease (CVD) represents the number 1 cause of death in type 2 diabetes (T2D) patients. This includes diabetic cardiomyopathy (DC), of which there are no approved therapies. Previous studies have shown that myocardial glucose oxidation rates are markedly impaired during T2D due to reduced pyruvate dehydrogenase (PDH) activity. Furthermore, forkhead Box O1 (FoxO1) activity is enhanced in T2D and has been shown to increase expression of PDH kinase 4 (Pdk4), which phosphorylates and inhibits PDH activity. Because the role of FoxO1 on glucose oxidation impairment during DC has not yet been assessed, our aim is to determine whether FoxO1 controls Pdhk4 transcription and its inhibition preserves PDH activity and glucose oxidation in the heart.

Methods: Differentiated C2C12 and H9c2 myotubes and myocytes, respectively were treated with AS1842856 (FoxO1 inhibitor) and/or Dexamethasone (FoxO1 activator) for 24 hrs and Pdk1/2 and 4 mRNA, protein expression and PDH phosphorylation status were evaluated. In addition, C57BL/6J mice were either fasted or followed by refeeding period, and extracted heart, muscles were evaluated for above mentioned mRNA and proteins. To examine the role of FoxO1 in regulation of the Pdk4 promoter, luciferase assays were performed.

Results: FoxO1 inhibition in C2C12 myotubes significantly decreased Pdk4 mRNA (~50%) and PDHK4 protein expression, which correlated with a significant decrease in PDH phosphorylation. Likewise, similar observations were seen in H9c2 myocytes. Dexamethasone induced Pdk4 mRNA in both C2C12 myotubes and H9c2 myocytes, which was markedly attenuated via pretreatment with AS1842856, and these findings translated to the appropriate changes in PDHK4 protein expression and PDH phosphorylation. As fasting is known to activate FoxO1, 4 hrs re-feeding after 16 hrs fasting in mice leads to a significant decrease (~60%) in Pdk4 mRNA expression in muscle compared to mice fasted for 20 hrs. The decrease in Pdk4 mRNA level was further corroborated by a decrease in PDH phosphorylation. Furthermore, luciferase activity assays showed significant upregulation of Pdk4 promoter activity by FoxO1-ADA (active) and downregulation by FoxO1-D256 (dominant negative) when compared to FoxO1 WT in H9c2 myocytes.

Conclusion: Our results suggest that FoxO1 controls Pdk4 transcription in the heart, and that it may regulate PDH activity and glucose oxidation. We are currently eliminating both FoxO1 and PDH specifically in the heart to delineate their roles in the pathogenesis of DC.

Keywords: FoxO1; Pyruvate dehydrogenase, Glucose oxidation; Diabetic cardiomyopathy.
Androgens Modulate Lipid Metabolism and Lipidogenic pathways In a Rodent Model of Metabolic Syndrome and Polycystic Ovary Syndrome.

Gayathri Ananthakrishnan, Spencer Proctor, Mahua Ghosh, Rene Jacobs and Donna Vine

Metabolic & Cardiovascular Disease Laboratory, Department of Agriculture, Food and Nutritional Science, University of Alberta

**Background:** In polycystic ovary syndrome (PCOS) a high plasma level of testosterone (T) has been correlated with an adverse plasma lipid profile and exacerbated CVD risk. At present we do not know the physiological or mechanistic pathways of how androgens regulate lipid metabolism under control or PCOS conditions. Previous studies from our laboratory have shown that flutamide, an androgen receptor (AR) inhibitor, reduces plasma concentration of triglycerides (TG) and apoB-lipoproteins, and intestinal secretion of TG. The aim of this study was to determine the direct effects of androgens, T and dihydrotestosterone (DHT), on lipid metabolism in a rodent model of metabolic syndrome (MetS) and PCOS.

**Methods:** Control and PCOS-MetS rodents were administered vehicle, T or DHT (non-aromatizable) for 7 days. Following treatment animals underwent a mesenteric lymphatic cannulation procedure to determine effects on intestinal chylomicron (apoB48) and lipid secretion, and absorption using radiolabelled [³H]-cholesterol and [¹⁴C]-palmitic acid. Plasma and lymph lipids were measured using calorimetric assays and hepatic/intestinal mRNA expression of select genes was determined using RT-PCR.

**Results:** T and DHT treatment increased plasma free T and reduced SHBG concentration in control and PCOS-prone animals. Plasma LDL-C was increased with DHT treatment in control and both T & DHT in PCOS-MetS groups, with no effect on other plasma lipids. Intestinal TG secretion and absorption of TG and cholesterol were increased in T and DHT treated PCOS-MetS animals only. Whereas DHT reduced intestinal apoB48 secretion in both control and PCOS-MetS animals. These differential effects of T and DHT were associated with changes in lipidogenic genes (SREBP1a, SREBP1c, SREBP-2, ACC, MTP, and LXRα) and steroidogenic genes (AR, ER and SRD5A1) in the liver and intestine.

**Conclusion:** These results show androgens modulate intestinal and hepatic pathways in the synthesis and absorption of lipids, and T and DHT differentially affect intestinal chylomicron secretion and lipid absorption in PCOS-MetS conditions. In conclusion, these results suggest that androgens cause or exacerbate lipid and lipoprotein metabolism in PCOS.

**Key words:** Polycystic ovary syndrome (PCOS), metabolic syndrome (MetS), lipid metabolism, testosterone, dihydrotestosterone.
STRATEGIES FOR COMPLEX INTERVENTION IMPLEMENTATION IN PRIMARY CARE: THE INTERACTIVE PROCESS FRAMEWORK AND THE 5AS TEAM OBESITY STUDY

Thea Luig, Jodie Asselin, Arya M Sharma, Denise L Campbell-Scherer
5As Team Project, Department of Medicine/Family Medicine, Faculty of Medicine & Dentistry, University of Alberta

Background: Knowledge transfer in complex practice settings is challenging. Although several theoretically informed frameworks for implementation and dissemination of new knowledge into clinical practice exist, there is a lack of understanding of the complex processes and interactions that impact implementation and outcomes. The 5As Team intervention (5AsT) aimed to improve quality and quantity of weight management provided by interdisciplinary clinicians at a large Primary Care Network (PCN) in Edmonton, Alberta, and made an explicit effort to understand the implementation process, interactions with context, and impacts on outcomes. This work reports the findings on the 5AsT implementation strategy as they help to further develop the Interactive Systems Framework (ISF), a framework for dissemination and implementation. Results illuminate the interactions between the framework’s components.

Methods: Secondary qualitative analysis of the implementation process of 5AsT, a randomized control trial with convergent mixed-methods evaluation. Review of 61 models and frameworks for dissemination and implementation.

Results: Increase in interdisciplinary relationships, communication, and confidence emerged as both an intervention outcome and a facilitator of implementation success. Dynamic design of iterative evaluation and flexible implementation strategy proved crucial for sustainable, context-appropriate intervention impact. Collaboration with PCN staff and management throughout the project ensured a positive implementation environment and the transformation of evidence into knowledge-in-practice-in-context. While findings support the focus on collaboration, capacity building, and communication proposed by the Interactive Systems Framework, they also illuminate under-researched processes and interactions that impact implementation processes. The Interactive Process Framework for the implementation of complex interventions is proposed.

Conclusion: Sustained engagement that respects tacit knowledge and contextual expertise, as well as dynamic evaluation and intervention design proved effective for multi-directional knowledge exchange and enabled the co-creation of contextually relevant knowledge-in-practice.

Keywords: Implementation, complex interventions, theoretical framework, primary care, obesity management
CHARACTERIZING AND OPTIMIZING PSEUDOISLETS

Yang Yu, Mark Ungrin

Biomedical Engineering Graduate Program, University of Calgary

Background: Islets transplantation is a promising approach to the treatment of Type I Diabetes. However, a major clinical limitation for current islet transplantation is the inefficient survival and engraftment in the immediate post-transplant period. One reason is likely that the size of the native islets is too large for sufficient oxygen and nutrition delivery in avascular condition immediately after transplant. Quantitative modeling of oxygen delivery within islets shows significant benefits to smaller islets, and consistent with this concept, smaller human islets have been reported to perform better than larger ones in a clinical setting. Based on our previous experience in microtissue formation, I hypothesize that there exists an optimal aggregate size, at which islet cell aggregates will exhibit significantly improved viability and insulin secretion capacity, due at least in part to improved oxygen transport properties. I further hypothesize that the pseudoislet formation process can be modulated by soluble signals allowing further increases in viability and function. Subsequently, the optimal outcome from in vitro experiments will be validated in vivo. I hypothesize that in vitro results will be generally predictive of and consistent with in vivo survival and function.

Methods: The Aggrewell system is used to re-aggregate the native islets to form smaller pseudoislets and centrifugation is employed to accelerate aggregation and enhance viability. Re-aggregated islet cells were cultured in supplemented CMRL-1066 for 48hrs in order to form pseudoislets. Insulin production capacity is tested in Glucose Stimulated Insulin Secretion (GSIS) assay at multiple time points. Design of experiments (DoE) is used for design and analysis of in vitro optimization.

Results: In the recent preliminary results, I successfully re-aggregated the native islet cells to formed pseudoislets in our centrifugation-aided microwell system by using neonatal porcine islets (NPI) and human donor islets respectively. The direct comparison showed that the pseudoislets significantly outperformed the native islets after 48-hour incubation, exhibiting a 2.8 fold increase in terms of insulin secretion per input cell basis (p<0.02). This effect is further improved by an additional 1.6- and 2.5-fold increase when performing the experiment in the presence of a ROCK inhibitor Y27632, or the apoptosis inhibitor Emricasan (p<0.05; p<0.01). In another independent experiment, direct comparison with native islets shows a 2.7- and 3.7-fold increase in ER (p<0.003) with Emricasan or Emricasan plus Y27632. The RSM model generated from a two-factorial (size and ibuprofen concentration) pilot experiment recently gives a R-square of 0.897, with the optimum point at size 217 and Ibuprofen concentration 15.56 uM indicating a relatively good fit.

Conclusion: Overall the new approach to the generation of pseudoislets is feasible. If consistent results are obtained in vivo this would result in at least a 3.7-fold increase in the number of patients treatable from the current limited supply of donor islets. Moreover, as approximately 50% of islet isolations yield quantities that are insufficient for therapeutic applications, a successful clinical translation of the proposed research would also effectively expand the human islet donor pool, mitigating the current critical islet shortage for patients awaiting transplantation.

Keywords: Pseudoislets, Type I diabetes, Micro-tissue engineering, design of experiments
Kv1.2 CHANNELS AT THE INTERFACE OF REDOX AND ELECTRICAL EXCITABILITY

Victoria A. Baronas, Yury Y. Vilin, Runying Y. Yang, Harley T. Kurata.
Department of Anaesthesiology, Pharmacology and Therapeutics, University of British Columbia
Department of Pharmacology, University of Alberta

Background:
Oxidation and reduction of proteins influences function, and oxidative species play an important role in the progression of diabetes and its many complications. We have identified a molecular mechanism for sensing extracellular redox state and translating this into altered activity of voltage gated potassium channels (Kv). Within this diverse Kv channel family, Kv1.2 channels have a unique phenotype that sets them aside from all other Kv channels. They are subject to a regulatory mechanism referred to as 'use-dependent activation', that allows channels to progressively increase activity during trains of repetitive stimuli. We hypothesize that this regulatory mechanism is extrinsic to the Kv1.2 channel and demonstrate how it acts in a redox-dependent manner to modulate potassium channel function.

Methods:
Using patch clamp electrophysiology, we demonstrate the use-dependent behavior of the Kv1.2 channel and how it can be affected by redox. To identify regulatory binding partners, we have used a co-immunoprecipitation approach to isolate nearby proteins and mass spectrometry to identify them.

Results:
We demonstrate that use-dependent activation is a property that is unique to Kv1.2. Furthermore, when cells expressing Kv1.2 are exposed to reducing conditions, they become extremely susceptible to use-dependent activation such that a train of depolarizations will elicit a 100-fold plus increase in current. We show that this effect is specifically due to the extracellular redox environment, and that the response is not mediated by modification of cysteines intrinsic to the Kv1.2 channel, but rather an extrinsic redox-sensitive regulator.

Conclusions:
Kv1.2 channels are uniquely susceptible to high frequency trains of stimuli in a redox-dependent manner. Therefore, Kv1.2 stands at the interface of redox and electrical activity, able to tune potassium currents in response to changes of extracellular redox conditions.

Key words: Potassium channel, redox environment, epilepsy.
TRANSPLANTATION OF HUMAN DERIVED PANCREATIC ENDODERM CELLS REVERSED DIABETES POST-TRANSPLANTATION INTO A PREVASCULARIZED SUBCUTANEOUS SITE

Andrew R. Pepper, Rena Pawlick, Antonio Bruni, Doug O’Gorman, Richard Yan-Do, Boris Galo-Lopez, John Wink, Yasmin Rafiei, Kieran Purich, Tatsuya Kin, Patrick MacDonald, A. M. James Shapiro

Departments of Surgery & Pharmacology, Alberta Diabetes Institute, University of Alberta

Background: Beta (β) cell replacement therapy is an effective means to restore glucose homeostasis in selected individuals with type 1 diabetes. The scarcity of ‘healthy’ human donor pancreata further restricts the broad application of this therapy. ‘β-like’ cells derived from human embryonic cells (hESC), have been developed in vitro. However, translation to clinical investigation, especially immune isolating approaches, has been limited by host immune response to foreign material. Herein, we examine the efficacy hESC derived pancreatic endoderm cells (PEC) to reverse diabetes post-transplantation into the prevascularized subcutaneous ‘device-less’ (DL) site.

Methods: Succeeding a month catheter implant period, chemically induced diabetic B6/Rag-/- mice were transplanted with 10-20μL (~0.5-1.0 x 10^7 cells) of PEC (ViaCyte, San Diego, CA). Recipient mice were randomly distributed into three transplant groups: 1) epididymal fat pad (FP), 2) subcutaneous alone (SC), or 3) DL. Post-transplant function was assessed through twice-weekly non-fasting blood glucose measurements, human C-peptide secretion and intraperitoneal glucose tolerance testing (IPGTT).

Results: Transplant PEC were able to reverse diabetes in 33% (2/6) of the recipients in both FP and SC groups, with a mean time to euglycemia of 110.5 ± 10.5 and 116.0 ± 19.0 days, respectively. In contrast, in the DL group, 100% of the mice (22/22) transplanted with PEC became normoglycemic within 99.8 ± 3.8 days (p<0.01, p<0.01 respectively, log-rank). Furthermore, PEC-DL transplanted mice demonstrated glucose responsive human C-peptide secretion (p<0.01, paired two-tail t-test). In response to IPGTT, PEC-DL mice demonstrated glucose clearance profiles similar to non-diabetic controls. Glucose homeostasis was abolished upon graft excision. Retrieved grafts stained positive for insulin, glucagon and somatostatin.

Conclusion: This advancement mitigates chronic foreign body response evoked by biomaterials, utilizes a device- and growth factor-free approach, facilitates in vivo differentiation of PEC into mature, glucose-responsive insulin-producing cells, and restores glycemic control in a reliable and effective manner.

Keywords: Stem cell, diabetes, transplantation
MAJOR URINARY PROTEIN: UNEXPECTED EXPRESSION AND ROLES IN DIABETIC PERIPHERAL NEURONS

Trevor Poitras, Vandana Singh, Ambika Chandrasekhar, Jose A Martinez and Douglas W Zochodne. Neuroscience and Mental Health Institute, Alberta Diabetes Institute and, Division of Neurology, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2R3

Background: An array gene expression analysis of the dorsal root ganglion of long term diabetic mice identified novel upregulation of major urinary protein mRNAs (MUP 1, 2). These barrel shaped proteins were originally postulated to play a role in secretion of pheromones in rodents, but had unexpected expression in adult rat and mouse sensory neurons. Their upregulation in experimental diabetes was investigated. Chronic type 1 diabetic mice (3-4 months duration; streptozotocin (STZ)) exhibit a decline in nerve conduction velocity (NCV) in both sensory and motor nerves, and an increase in withdrawal latency when presented with thermal stimulation, features of experimental diabetic polyneuropathy (DPN). The impact of MUP2 knockdown was investigated on the development of DPN.

Methods: Adult mice were treated using STZ to generate a chronic diabetes phenotype. Hyperglycemia was confirmed through blood glucose testing three months following induction. Mice were administered MUP siRNA through intranasal injection 3 times a week for 3 weeks. Multifiber electrophysiological recordings of motor and sensory fibers were carried out to evaluate the sensory and motor NCV. Thermal testing was performed using a Hargreave’s thermal testing protocol under the hind paws of the mice measuring the latency of withdrawal.

Results: Diabetic mice had an expected decline in motor and sensory NCV. However once treated with MUP siRNA, the diabetic mice showed no difference when compared to the control mice treated scrambled siRNA in motor and sensory NCV. Thermal latency showed a nonsignificant trend towards improvement in diabetic mice treated with MUP siRNA. The improvements in electrophysiological indices of DPN correlated with an enhancement of neurite outgrowth in adult sensory neurons undergoing MUP1,2 knockdown in vitro.

Conclusion: These findings suggest that aberrant upregulation of an unexpected sensory pheromone within sensory neurons acts to suppress their growth properties. In experimental diabetes, its knockdown improves features of DPN.

Supported by CIHR, CDA.

Keywords: Electrophysiology, MUPs, Polyneuropathy
IMPACT OF DRUG EXPOSURE DEFINITIONS ON OBSERVED ASSOCIATION IN PHARMACOEPIDEMIOLOGY RESEARCH

Maxim Eskin, Eurich Dean, Scot Simpson

Alliance for Canadian Health Outcomes Research in Diabetes (ACORD), School of Public Health, University of Alberta

Background: Accurate assessment of medication use is an essential component of any pharmacoepidemiologic research as misclassification of exposure will threaten study validity and lead to spurious associations. Many pharmacoepidemiological studies, however, use crude definitions, such as the categorical “any or no use” to classify exposure, which has potentially serious drawbacks. This approach has led to numerous highly publicized observational studies of the effect of diabetes medications on health outcomes reporting exaggerated relationships that were later contradicted by randomized controlled trials. Rectifying the differences observed in pharmacoepidemiological studies of metformin to RCTs is difficult. Although numerous factors (e.g., selection bias, unmeasured confounding) likely play a significant role, in many studies a key underlying factor, which is often overlooked, is the method to define the exposure. It is possible some, if not all, of the benefit, observed with metformin in observational studies may be related to analytic design and exposure definitions.

Methods: New users of oral anti-hyperglycemic drugs were identified using the administrative databases from Alberta between 1998 and 2010. Metformin exposure was described using 10 definitions commonly used in cohort and case-control studies. All analyses included the same covariates of age, gender, and a co-morbidity score. The association between metformin exposure and all-cause mortality was assessed using a Cox Proportional Hazards model for cohort studies and conditional logistic regression analysis for case-control studies. For all analyses, patients not receiving metformin served as the reference group.

Results: Overall, we identified 64,293 new oral anti-hyperglycemic drugs users. Mean age was 68.9 years, 33,131 (52%) were male, 55,525 (86%) had at least 1 prescription claim for metformin, and 24,745 (39%) of patients died during an average follow-up of 5.8 years. In adjusted models, the association between metformin and mortality varied widely ranging from 75% reduced risk of mortality (aHR 0.25, 95% CI 0.24-0.26) to 29% increased risk (aHR 1.29, 95% CI 1.25-1.33); however, most metformin exposure definitions provided estimates in the 0.6-0.8 range aligning with the estimates of previous observational studies.

Conclusions: We observed a wide range of associations between mortality risk and different exposure definitions. Since no exposure definition is completely free of bias pharmacoepidemiological studies should consider using at least two exposure definitions as well as sensitivity analyses of exposure definitions to provide more robust and potentially valid study estimates.

Keywords: Exposure definition, Metformin, Pharmacoepidemiology
REGULATORS OF GLYCINE SIGNALING IN HUMAN PANCREATIC BETA CELLS

Richard Yan-Do, Kunimasa Suzuki, Mourad Ferdaoussi, Cathy Hajmrle, Patrick E. MacDonald

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

Background: Glycine is an inhibitory neurotransmitter which opens a family of ligand gated chloride channels called glycine receptors. Glycine receptors are expressed on human pancreatic beta cells and previous studies suggest that glycine is a potential biomarker for type 2 diabetes (T2D). A strong correlation exists between plasma glycine concentrations and insulin sensitivity, glucose disposal, and obesity. Unlike the CNS, glycine in the pancreas is excitatory, and can stimulate insulin secretion by directly activating glycine receptors in human β-cells. We seek to examine how glycine regulates insulin secretion and what factors regulate glycine signaling in human islets.

Methods: Human islets were isolated in the Alberta Diabetes Institute Islet Core and the Clinical Islet Laboratory at the University of Alberta from donor organs. Electrical recordings and calcium imaging was performed on dispersed human islets from healthy donors and donors with T2D.

Results: Glycine receptors were identified on both α and β cells in human islets and quantitative immunofluorescence determined that glycine receptor expression is decreased in T2D islets (from 480 ±35 average pixel intensity in donors without T2D to 309±24 average PI in donors with T2D). Glycine receptor α1 subunit mRNA is elevated in donors with T2D while Glycine receptor α3 and β subunit are not significantly different from donors without T2D. Application of 0.3mM glycine was found to produce 27±6pA/pF current in healthy human β cells whereas it was significantly lower in T2D β cells (16±2pA/pF). Glycine current amplitude was found to be inversely proportional to donor HbA1C while no correlation was observed between glycine current amplitude and donor BMI. Culturing dispersed human islets from donors without diabetes in high glucose (20mM) conditions was found to impair glycine current in a reversible manner. Downstream of the receptors, application of 0.3mM glycine resulted in depolarization of β cells (16±6mV), elevation of intracellular calcium (0.06±0.01 arbitrary units), and stimulation of insulin secretion. Interestingly, glycine receptor activity was found to be regulated by insulin and PI3K, however the mechanism by which this occurs is not clear. Preliminary evidence suggests post-translational modification of glycine receptor trafficking proteins may be involved.

Conclusion: Here we have evidence that glycine receptor expression is dynamic and factors such as glucose, insulin, or the presence of T2D can influence the glycine receptor expression in human islets. Insulin autocrine feedback in human β-cells amplifies the glycine-mediated current but this effect is not observed in donors with T2D. Finally, individuals with T2D demonstrate an impaired glycine receptors expression and glycine signaling profile.

Keywords: glycine receptors, Type 2 diabetes, human islets
ADROPIN ENHANCEMENT OF CARDIAC INSULIN SENSITIVITY AND INHIBITION OF FATTY ACID OXIDATION ARE ASSOCIATED WITH IMPROVEMENT OF CARDIAC FUNCTION AND EFFICIENCY

Tariq Altamimi, Arata Fukushima, Liyan Zhang, Su Gao, Abhishek Gupta, Gary D. Lopaschuk.

Cardiovascular Research Centre, University of Alberta.

Background: Impaired cardiac insulin signaling and high cardiac fatty acid oxidation rates are characteristics of diabetic cardiomyopathy (DCM). Potential roles for liver-derived metabolic factors in mediating cardiac energy homeostasis are underappreciated. Plasma levels of adropin, a liver secreted peptide, increase during feeding and decrease during fasting and in diabetes. In skeletal muscle, adropin preferentially promotes glucose over fatty acid oxidation. We therefore aimed at determining what effect adropin has on cardiac energy metabolism, insulin signaling and cardiac efficiency.

Methods: In vivo: mice were given intra-peritoneal injections of adropin (450 nmol/kg) or vehicle to for 3 times over 20-24 hrs. with a 16-20 hr-fasting (to accentuate the differences in adropin plasma levels between the two groups and to simulate the status of higher fatty acid oxidation seen in the diabetic heart) prior to ex vivo working heart perfusion for 30 min without insulin, followed by 30 min with 100 μU/mL insulin. Acute: To rule out acute adropin metabolic/functional effects on the heart not depending on protein expression, we perfused non-fasting mouse hearts in the presence of 100 μU/mL insulin with or without 2nM adropin. Glucose and palmitate oxidation were measured by quantitative collection of 14CO2 and 3H2O produced by oxidation of [U-14C]glucose and 0.8 mM [9, 10-3H]palmitate, respectively. Immunoblotting was used to analyze expression/ phosphorylation of key metabolic proteins.

Results: Despite fasting-induced predominance of fatty acid oxidation, insulin inhibition of fatty acid oxidation was re-established in hearts from adropin-treated mice (from 1022±143 to 517±56 nmol · g dry wt⁻¹ · min⁻¹, p <0.05) compared to vehicle-treated mice (from 757±104 to 818±103 nmol · g dry wt⁻¹ · min⁻¹). Adropin-treated mouse hearts showed higher cardiac work over the course of perfusion (p<0.05), which was accompanied by improved cardiac efficiency and enhanced phosphorylation of insulin signaling proteins (tyrosine-IRS-1, AS160, p<0.05). Acute adropin administered to ex vivo hearts showed a robust stimulation of glucose oxidation compared to controls (3025±401 vs 1708±292 nmol · g dry wt⁻¹ · min⁻¹, p<0.05, respectively) with a corresponding inhibition of palmitate oxidation (325±61 vs 731±160 nmol · g dry wt⁻¹ · min⁻¹, p<0.05, respectively). Acute adropin also increased IRS-1 tyrosine-phosphorylation as well as Akt, and GSK3β phosphorylation (p<0.05), suggesting acute receptor- and/or post-translational modification-mediated mechanisms.

Conclusion: These results suggest adropin to modulate cardiac metabolism favoring glucose utilization and improving insulin sensitivity and propose it as an option for the treatment of DCM.

Keywords: Adropin, Cardiac Metabolism, Insulin Signaling, Diabetic Cardiomyopathy.
PREVALENCE AND INCIDENCE OF DIABETES IN ALBERTA'S TOMORROW PROJECT COHORT – LINKAGE WITH ADMINISTRATIVE HEALTHCARE DATA

Ming Ye1, Paula J. Robson2, Dean T. Eurich1, Jennifer E. Vena2, Jian-Yi Xu2, Jeffrey A. Johnson1

1 School of Public Health, University of Alberta; 2 Alberta’s Tomorrow Project, CancerControl Alberta, Alberta Health Services; 3 Department of Agricultural, Food and Nutritional Science, University of Alberta

Background: The prevalence of diabetes in Alberta is estimated to increase from 7.0% in 2014 to 9.6% in 2024. Alberta’s Tomorrow Project (ATP) is a province-wide cohort study of adult Albertans aged 35-69 years to support research on cancer and other chronic diseases, such as diabetes. To accurately estimate the prevalence and incidence of diabetes among the ATP cohort for future etiological study, cohort data were linked to Alberta Health (AH) administrative healthcare databases.

Methods: Individual-level data of a total of 52,851 ATP participants were linked to AH administrative healthcare datasets for complete follow-up time (Oct 2000-Mar 2015) with participants’ consents to using personal health numbers for data linkage. Cases of diabetes were identified using the National Diabetes Surveillance System (NDSS) algorithm for administrative data- one hospitalization with an ICD-9 or -10 code of diabetes in the hospital discharge abstract database or two physician claims within two years with an ICD-9 code of diabetes in provider claims database. An additional algorithm for identifying prevalent cases was developed using less stringent criteria (“non-NDSS”), which was self-report of diabetes at enrollment plus any of the following: i) one hospitalization with an ICD-9 or -10 code of diabetes, ii) one physician claim with an ICD-9 code of diabetes, or iii) one diabetes medication with ATC code of insulin (A10A) or glucose-lowering drugs (A10B) from either the Pharmaceutical Information Network or Alberta Blue Cross dataset. Cases were identified as “incident” if the NDSS index date of diabetes was >6 months after the ATP enrollment date.

Results: Within the ATP cohort, there were 4,926 cases of diabetes identified using NDSS (n=4,685) or non-NDSS (prevalent cases only, n=2,415) criteria. The prevalence of diabetes was 5.7% (n=3,013) at enrollment; during the course of follow-up time, 1,913 participants developed diabetes and the corresponding incidence rate was 5.6%. Compared to administrative healthcare data, ATP self-report data at enrollment had relatively high sensitivity (78.1%) and specificity (98.4%) in identifying prevalent cases of diabetes.

Conclusion: By linking with administrative healthcare data, we were able to estimate the prevalence and incidence of diabetes in the ATP cohort. In this cohort, questionnaire-based self-report corresponded very well to the objectively assessed administrative data, and therefore can be a valid source for assessing diabetes prevalence.

Key words: diabetes prevalence, diabetes incidence, Alberta’s Tomorrow Project, healthcare data
A MINIMUM CONDITIONING PROTOCOL TOWARDS TRANSPLANTATION TOLERANCE IN NOD MICE BY MIXED HEMATOPOIETIC CHIMERISM

Jiaxin Lin, William F. N. Chan, Colin C. Anderson

Department of Surgery, Faculty of Medicine and Dentistry, University of Alberta

**Background:** Stable mixed hematopoietic chimerism is a robust method for generating donor specific tolerance with the potential to allow islet transplant tolerance in diabetic recipients. However, its clinical application is prevented by the toxicity of current recipient conditioning regimens (e.g., use of irradiation). We previously showed that an irradiation-free mixed chimerism protocol in diabetes prone NOD mice is achievable with antibodies to T cells and CD40L together with busulfan and high dose rapamycin. We sought to generate a more clinically feasible chimerism protocol and tested the hypothesis that more efficient recipient T cell depletion would eliminate the need for anti-CD40L (known to cause thromboembolism in humans) and rapamycin.

**Methods:** We preconditioned NOD mice with donor specific transfusion (DST) from fully mismatched C3H or FVB mice (day -10), cyclophosphamide (day -8), anti-T-cell antibodies against CD90 and/or CD4 + CD8 (day -6, -1, 4, 9, 14), and busulfan (day -1). Donor bone marrow transplantation (BMTx) was done at day 0. Body weight and blood glucose levels of recipient mice were assessed weekly. Flow cytometry was used to detect chimerism and different lineages of cells from recipient and donor mice.

**Results:** By using this protocol, we successfully induced mixed chimerism in 28/38 NOD mice with the level of donor cells up to 90%. Stable chimerism with multi-lineage donor cells, including T, B, NK and Dendritic cells was maintained in 16/27 recipients. Loss of chimerism could be predicted by a lower early level of chimerism at 4, 9 or 14 days post BMTx. We determined that inclusion of anti-CD90 mAb in the conditioning regimen could significantly accelerate the depletion of recipient T cells and facilitate stable chimerism. The loss of anti-donor V beta 11+ T cells in stable C3H to NOD chimeric mice and anti-recipient V beta 17+ T cells in stable FVB to NOD chimeric mice indicated the establishment of chimerism involves clonal deletion. 5/5 chimeric mice accepted engrafted donor skin from bone marrow cell donors but rejected skin from MHC-matched and minor antigen mis-matched donors, which suggests the chimeric mice are immunocompetent and tolerant to donor.

**Conclusions:** A rapid and robust recipient T cell depletion protocol generated chimerism without the need for anti-CD40L or rapamycin. This protocol is the most clinically feasible to have achieved fully allogeneic mixed chimerism in NOD mice.

**Key words:** Hematopoietic chimerism, Transplantation tolerance, Bone marrow transplantation
ALTERED DIETARY LIPID METABOLISM IN A NOVEL MOUSE MODEL WITH INTESTINAL-SPECIFIC DELETION OF CTα

John P. Kennelly, Jelske van der Veen, Randy Nelson, Robin da Silva, Kelly-Ann Leonard, René Jacobs

Department of Agricultural, Food and Nutritional Sciences, University of Alberta

**Background:** Phosphatidylcholine (PC) is produced from dietary choline by the CDP-choline (Kennedy) pathway in all cells, and the enzyme CTP: phosphocholine Cytidylyltransferase (CT) regulates flux through the pathway. CTα is an important regulator of hepatic lipoprotein metabolism. Furthermore, PC biosynthesis is important for maintaining the structural integrity of membranes and for facilitating the incorporation of fatty acids into lipid droplets. Therefore, CTα may play a role in intestinal lipid metabolism. To determine the functional importance of CTα and PC in regulating intestinal lipid and lipoprotein metabolism in vivo, we have generated intestinal-specific CTα deficient mice (iCTα-/-) mice using a tamoxifen-inducible Cre-Lox system.

**Methods:** Experiments were conducted in the setting of either a chow diet or 40% kilocalories fat diet. Blood and intestinal tissue was collected for lipid, transcriptional and histological analysis. Chylomicron secretion was assessed by administering mice with an oral bolus of olive oil and measuring appearance of triglyceride in plasma at various time-points.

**Results:** When maintained on chow diet, iCTα-/- mice had normal fasting plasma lipid levels and had normal chylomicron secretion as compared to controls. When placed on a 40% high fat diet, iCTα-/- mice experienced dramatic weight loss after one week, at least partly attributed to inhibition of food intake. 5-day high fat diet-fed iCTα-/- mice had decreased plasma triglycerides, cholesterol and chylomicron secretion rate as compared to controls. Furthermore, intestinal triglyceride levels were decreased in iCTα-/- mice compared to controls, suggesting lipid absorption was impaired.

**Conclusion:** These findings suggest CTα plays an important role in regulating intestinal lipid metabolism. Future studies will investigate the mechanisms behind altered lipid metabolism in iCTα-/- mice.

**Keywords:** lipids, metabolism, intestine.
IMPROVED GLUCOSE HOMEOSTASIS IN OBESE MICE IS ASSOCIATED WITH RESVERATROL-MEDIATED ALTERATIONS IN THE GUT MICROBIOME

Ty T. Kim1, Miranda M. Sung1, Emmanuel Denou2, Carrie-Lynn M. Soltys1, Shereen M. Hamza1, Nikole J. Byrne1, Grant Masson1, Heekuk Park3, David S. Wishart4, Karen L. Madsen3, Jonathan D. Schertzer2 and Jason R. B. Dyck1,*

1Department of Pediatrics, Faculty of Medicine & Dentistry, U of Alberta; 2Department of Biochemistry and Biomedical Sciences, Faculty of Health Sciences, McMaster U; 3The Metabolomics Innovation Centre, U of Alberta; 4Department of Medicine, Faculty of Medicine & Dentistry, U of Alberta.

Background: Resveratrol is a bioactive polyphenol with antifungal/antimicrobial qualities that has shown promising results in the prevention and/or treatment of insulin resistance and type 2 diabetes. Although oral administration of resveratrol is able to improve glucose homeostasis in obese individuals, resveratrol has low bioavailability and largely arrives unmetabolized in the colon. Given that drugs and therapies can exert effects through combined actions on the host and microbiota, it has been suggested that resveratrol may improve glucose homeostasis through alteration of the gut microbiome. Since obesity and metabolic disease are also associated with changes in the gut microbiota, we sought to characterize the effects of resveratrol ingestion on the taxonomic and functional changes of the gut microbiota in a mouse model of diet-induced obesity.

Methods: To confirm that resveratrol supplementation improves glucose homeostasis, conventional raised 8 week-old C57Bl/6 mice were maintained on a Chow (±resveratrol) or high fat-high sucrose (HFHS) (±resveratrol) diet (0.4% resveratrol diet ad libitum) for 8 weeks to induce impaired glucose homeostasis. Furthermore, to address whether resveratrol-induced changes in gut microbiota are a potential mechanism by which resveratrol improves glucose homeostasis, fecal matter from mice fed a Chow (±resveratrol) diet were used for fecal microbiota transfer (FMT) in mice subjected to a HFHS diet for 6 weeks.

Results: HFHS-fed conventional mice supplemented with resveratrol showed improved glucose tolerance and peripheral insulin signaling in comparison to mice maintained on a regular HFHS diet. Associated with this resveratrol ingestion produced taxonomic and predicted functional changes in the gut microbiome in obese mice. Moreover, FMT from resveratrol-fed donor mice to obese mice is sufficient to improve glucose tolerance and peripheral insulin signaling within 11 days of the first transplant.

Conclusion: These findings demonstrate that FMT from resveratrol-fed mice is sufficient to mimic the effects of an oral treatment of resveratrol, and highlight the potential importance of metabolites of resveratrol or bacterial-derived metabolites as an integral mechanism by which resveratrol improves glucose homeostasis in obesity.

Key words: resveratrol, gut microbiome, glucose homeostasis
PURE PRAIRIE LIVING PROGRAM - COMMUNITY BASED LIFESTYLE INTERVENTION

Fatheema Subhan, Kevin Li, Julia MacLaren, Elaine St. Laurent, Robin Anderson, Catherine B Chan

Department of AFNS, University of Alberta and Edmonton Southside Primary Care Network

Background: In Canada, there is a growing burden of type 2 diabetes (T2D). Nutrition therapy is an essential component of diabetes treatment but appears to be challenging to adopt. Strategies to implement nutrition recommendations and promote sustainable eating behaviours are required. Our research team developed an educational curriculum and a menu plan/cookbook, “Pure Prairie Eating Plan”. It was designed using the 4-A Framework, which suggests a healthy dietary pattern by ensuring foods in the menu are locally Available, financially/physically Accessible, culturally Acceptable and nutritionally Adequate. The resources translate the Canadian Diabetes Association (CDA) nutrition therapy guidelines into a practical 4 week menu plan, provides practical information about Eating Well with Canada's Food Guide (EWCFG), portion control, label reading and other skills (Pure Prairie Living Plan, PPLP). PPLP promotes behaviour change and skill development among people with diabetes. An intervention using PPLP was effective with volunteers in a research setting but the suitability of PPLP in a community setting has not been tested yet. Thus the overall objective of this study was to use the REAIM (Reach, Effectiveness, Adoption, Implementation and Maintenance) framework to evaluate a pilot implementation of the PPLP in a community setting among people with T2D.

Method: The study was approved by the University of Alberta Research Ethics Board. Study participant recruitment and intervention was conducted at the Edmonton Southside Primary Care Network; 26 adults with T2D consented to participate in a 5-week lifestyle intervention. Participants came for pre and post intervention assessments at baseline and 3 months. Weight, height, haemoglobin A1C (HbA1c) and blood pressure (BP) were measured. Questionnaires to assess dietary adherence, diabetes self-efficacy (DES), physical activity and quality of life were completed. Focus group discussions to evaluate the barriers/ facilitators to the intervention were conducted. In this report, the A in REAIM (adoption/implementation) with respect to improving behaviour outcomes (dietary adherence, self-efficacy) and the E (efficacy) with respect to health indicators (weight, body mass index (BMI), BP and HbA1c are documented. Paired t test was conducted to assess changes in the outcome measures.

Results: Significant improvement (p< 0.05) in dietary adherence score (+SD) (48.9 ± 11.2 vs 53.6 ± 14.0; n=9), DES score (48.4 ± 15.0 vs 62.4 ±10.8; n=9), weight (101.8 ± 25.9 vs 97.6 ± 25.3 kg; n=17), BMI (36.5 ± 8.1 vs. 34.9 ± 8.0 kg/m²; n=17), Systolic BP (139.8 ± 20.9 vs. 125.6 ± 16.3 mm Hg; n=17) and Diastolic BP (83.4 ± 11.6 vs. 77.4 ± 11.3 mm Hg; n=17) were observed from baseline to 3 months.

Conclusion: The PPLP showed significant improvement in behaviour outcomes and health indicators. Follow-up assessments at 6 month period will shed light on long term effectiveness of the program.

Key words: Lifestyle intervention, 4-A framework, dietary adherence, haemoglobin A1C.
EXAMINING THE UTILITY OF BMX-001, A NOVEL REDOX-ACTIVE METALLOPORPHYRIN, IN A MURINE SYNGENEIC, MARGINAL ISLET TRANSPLANT MODEL

Antonio Bruni, Andrew R. Pepper, Rena Pawlick, Boris Gala-Lopez, and A.M. James Shapiro

Department of Surgery, Faculty of Medicine and Dentistry, University of Alberta

Background: Islet transplantation has become an accepted strategy to treat select patients with type-1 diabetes. However, islet loss during isolation and the acute post-transplant period may compromise long-term engraftment outcomes. The cellular mechanisms responsible for early islet loss have yet to be fully elucidated, though oxidative stress and inflammatory cascades contribute to graft attrition. The use of metalloporphyrin anti-inflammatory and antioxidant (MnP) compounds are an attractive therapeutic strategy to mitigate such events. Herein, we examine whether MnP administration during isolation and culture could improve in vitro and in vivo islet function.

Methods: Using a murine islet isolation model, BALB/c pancreata were distended with collagenase supplemented with 0, 10 or 34μM MnP. Subsequent to isolation, islet viability was assessed via static glucose-stimulated insulin secretion (sGSIS). Remaining islets were cultured for 24 hours in media supplemented with 0, 10 or 34μM MnP and subsequently assessed for sGSIS. In parallel to in vitro assessment, a marginal dose (150 islets) was transplanted under the renal capsule of diabetic BALB/c mice and assessed for post-transplant function through non-fasting blood glucose measurements and intraperitoneal glucose tolerance testing (IPGTT).

Results: Subsequent to isolation, sGSIS revealed no discernable difference between control and MnP-treatment groups. However, when assessed 24 hours post-culture, MnP treatment significantly improved sGSIS in a dose-dependent manner (p<0.05). Islet transplantation revealed improved restoration of euglycemia in MnP-treated islet recipients. 70% and 66% of 10 and 34μM MnP-treated islet recipients, respectively, became euglycemic as compared to 20% of control recipients. Both MnP treatment groups exhibited improved daily blood glucose profiles relative to non-treated control recipients.

Conclusions: 24 hour MnP treatment appears to improve in vitro islet function and in vivo engraftment outcomes in a murine, syngeneic marginal mass model. Further work evaluating the utility of MnP in islet isolation and transplantation may lead to improved clinical therapies.

Keywords: islet viability, Islet transplantation, metalloporphyrin, redox modulation
PEMT-DEFICIENCY IMPROVES HEPATIC INSULIN SIGNALING

Jelske N. van der Veen, Nicholas McCloskey, Susanne Lingrell, Morgan D. Fullerton, Dennis E. Vance and René L. Jacobs

Group on the Molecular and Cell Biology of Lipids, Department of Biochemistry, Faculty of Medicine and Dentistry

Background - Phosphatidylethanolamine N-methyltransferase (PEMT) is an important enzyme in hepatic phosphatidylcholine (PC) biosynthesis. It mediates the methylation of phosphatidylethanolamine (PE) to produce PC and contributes 30% of total hepatic PC synthesis. We discovered that Pemt⁻/⁻ mice fed a high-fat diet are protected from diet-induced obesity and whole-body insulin resistance, as measured by an insulin tolerance test. However, Pemt⁻/⁻ mice also develop severe non-alcoholic steatohepatitis (NASH).

Methods - Since NASH is often associated with hepatic insulin resistance, we investigated whether the increased insulin sensitivity was restricted to non-hepatic tissues or whether the liver was also insulin sensitive. Therefore, we performed hyperinsulinemic-euglycemic clamp studies in Pemt⁺/+ and Pemt⁻/⁻ mice fed the high-fat diet.

Results - Much to our surprise, the livers of Pemt⁻/⁻ mice were not insulin resistant, despite strongly elevated levels of hepatic triacylglycerols and diacylglycerols, as well as increased hepatic inflammation and fibrosis compared to Pemt⁺/+ mice. Endogenous glucose production was lower in Pemt⁻/⁻ mice under both basal and hyperinsulinemic conditions. Hepatic Pkcε was strongly reduced in Pemt⁻/⁻ livers. Additionally, the ratios p-Foxo1/Foxo1, p-Gsk3β/Gsk3β, but not p-Akt/Akt, were increased in Pemt⁻/⁻ mice, suggesting improved hepatic insulin signaling. These differences, however, were mainly due to reduced total levels of these proteins, possibly as a result of metabolic alterations in these high-fat diet-fed mice. Therefore we investigated insulin signaling in hepatocytes in vitro. Experiments in primary hepatocytes from Pemt⁺/+ and Pemt⁻/⁻ mice, as well as in rat McArdle-RH7777 hepatoma cells stably transfected with an empty- or PEMT-containing vector, revealed improved insulin signaling in the absence of PEMT and protection from palmitate-induced insulin resistance. In cells lacking PEMT, the PC:PE ratio was strongly reduced and diacylglycerol levels were 50% lower than in cells expressing PEMT.

Conclusion - Thus, the phospholipid composition in hepatocytes seems critically important for insulin signaling. The reduced PC synthesis rate or the low PC:PE ratio in livers from Pemt⁻/⁻ mice protects against the development of lipid-induced hepatic insulin resistance.

Keywords – phospholipids, PEMT, insulin signaling, liver
ACTIVATION OF PKD1 BY AUTOCRINE ATP SIGNALING IN PANCREATIC β CELLS

Shara Khan, Mourad Ferdauossi, Valérie Bergeron, Nancy Smith, Austin Bautista, Patrick E. MacDonal.

Department of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta.

Background: ATP acts as a positive autocrine signal in β cells by activating P2Y1 receptors, stimulating electrical activity and coupling Ca2+ influx to Ca2+ release from ER stores. While it has been shown that ATP feedback activates purinergic P2Y1 receptors, resulting in activation of PLC and spatially restricted production of DAG, the downstream signaling that couples P2Y1 activation to enhance insulin secretion remains to be fully elucidated. Since DAG itself has been shown to activate Protein Kinase D1 (PKD1) to potentiate glucose stimulated insulin secretion, we hypothesize that autocrine ATP signaling activates downstream PKD1 to regulate insulin secretion.

Methods: To test whether autocrine signaling via ATP activates PKD1 in insulin secreting cells, western blotting was performed to study the dose-dependent, time-dependent, glucose-dependent, depolarization-induced and antagonist-induced activation of PKD1 in INS 832/13 insulinoma cells by measuring phosphorylation at Ser916 (which indicates activation of the protein). Insulin secretion was measured from intact PKD1 knockout islets and then quantified using MSD ELISA. Intact mouse islets were treated and then transmission electron microscopy was used to acquire images. Expression of PKD1 mRNA was analysed by RT-PCR.

Results: The P2Y1 receptor agonist, MRS2365, induces PKD1 phosphorylation at Ser916 in INS 832/13 cells at a concentration as low as 10 nmol/l and the maximal response is observed at 20 mins. Similarly, stimulation with glucose (10 mmol/l) or direct depolarization with KCl (30 mmol/l) causes activation of PKD1. A reduction in PKD1 activation was observed upon application of P2Y1 antagonist, MRS 2279 (1μmol/l). Insulin secretion was measured from isolated PKD1 KO mouse islets at 1 mmol/l and 10 mmol/l glucose in the absence and presence of 0.1 μmol/l MRS 2365 and low insulin release was observed in the PKD1 KO mouse islets of the high glucose groups. Therefore, activation via ATP stimulates insulin secretion via PKD1. Finally, there was decreased number of docked insulin granules upon MRS 2365 treatment in the electron micrographs of mouse islets, and RT-PCR analysis confirmed expression of PKD1 in human islets.

Conclusion: PKD1 has been suggested to regulate insulin secretion and might be pivotal in maintaining β cell function in diabetes. Activation of PKD1 by ATP reveals a novel pathway which affects insulin secretion and may prove valuable as the purinergic receptors are now being explored as therapeutic targets.

Key words: β cells, P2Y1, PKD1, ATP, Insulin
SELF-REPORTED PHYSICAL ACTIVITY AMONG WOMEN WITH A PREVIOUS GESTATIONAL DIABETES PREGNANCY

Nonsikelelo Mathe 1,2, Abdulrhman Alghamdi3, Margie Davenport4, Sonia Butalia5,6 Jeffrey A. Johnson1, Steven T. Johnson1,2
1 Alliance for Canadian Health Outcomes Research in Diabetes, School of Public Health University of Alberta, 2 Faculty of Health Disciplines, Athabasca University, 3 Faculty of Science, University of Alberta, 4 Faculty of Physical Activity and Recreation, University of Alberta, 5 Division of Endocrinology, University of Calgary, 6 Alberta Health Services

Background: Women who had Gestational Diabetes Mellitus (GDM) in pregnancy are at increased risk for type 2 diabetes compared to women without GDM. Lifestyle modification, such as increased physical activity (PA) may aid in reducing the risk type 2 diabetes after GDM. The Canadian Diabetes Association (CDA) recommends at least 150 minutes per week of moderate to vigorous physical activity in 10 minutes bouts to maintain health and reduce the risk for type 2 diabetes. In this study, self-reported physical activity in women who had GDM was reported and compared with guidelines set by the Canadian diabetes association (CDA) for physical activity.

Methods: Women previously diagnosed with GDM (n=48) participating in the Healthy Eating and Active Living for Gestational Diabetes (HEALD-GDM) trial (clinicaltrials.gov:NCT02483949) answered questions on social demographics, lifestyle and PA. Specifically, women were asked to write down the average number of times per week and time in each session spent in vigorous, moderate and light intensity PA using the Godin leisure time PA questionnaire. Descriptive statistics were used to describe and compare the women’s PA to CDA guidelines.

Results: Participants were on average 36.4 years of age [standard deviation (SD) 10.5], mean body mass index was 31.6 (9.6) kg/m2, and HbA1c 5.6 (0.5)%, waist circumference 99.0 (16.3) cm. The majority were married (96%) had college education or higher (69%), were of Caucasian ethnicity (67%), had a household income >$100000 annually (60%), and were employed full-time (44%). Participants reported vigorous PA 1.3 times per week lasting an average of 14.4 (18.1) minutes per session, moderate intensity PA 2.8 (2.4) times per week for an average 33.3 (37.5) minutes per session. Light intensity PA 4.3 (2.1) times per week for an average 50.4 (115.3) minutes per session. Most (67%) women did not meet guidelines for MVPA of 150 minutes per week (in 10 minute bouts) set by the CDA.

Conclusion: Women who previously had GDM in pregnancy may benefit from consultation on how to meet CDA guidelines for PA to reduce their risk for future type 2 diabetes.

Key words: Physical activity, Gestational diabetes mellitus, Type 2 diabetes, CDA guidelines, Self-Report
THE EFFECTS OF NATURALLY DERIVED FRUIT EXTRACTS ON THE VIABILITY OF NEONATAL PORCINE ISLETS

Eric Boivin, Wenlong Huang, Josue Rodrigues Silva, Gina R Rayat
Alberta Diabetes Institute, Dept. of Surgery, Faculty of Medicine and Dentistry, University of Alberta

Background: Islet transplantation is being considered as an alternative treatment for type 1 diabetes mellitus. However, islet efficiency is greatly decreased during the transplantation process due to, among other factors, oxidative stress leading to islet cell death. One way to reduce the oxidative stress is to treat the islets with an antioxidant compound pre-transplantation. Naturally derived fruit extracts from the Chinese bayberry (Myrica rubra) and the trifoliate orange (Poncirus trifoliata) such as cyanidin-3-O-glucoside (C3G), naringin, neohesperidin and bergamottin are known to have strong antioxidant properties. Thus, we evaluated the effects of these extracts on the viability of neonatal porcine islets.

Methods: Neonatal porcine islets (n=4) were isolated and cultured at 37°C, 95% air, 5% CO2 for seven to ten days in Ham's F10 media. The islets were then transferred into 24-well plates with each well containing 500 islet equivalent (IEQ) in Ham's F10 media. The islets were either left untreated, treated with Rapamycin, a drug to induce apoptosis, or with 10 fruit extracts (two bayberry extract mixtures, one pure bayberry extract, three pure citrus extracts and four citrus extract mixtures). Five hundred IEQ were also treated with H2O2, a reactive oxygen species, two hours before collection to induce necrosis. After 24 hours the cells were collected and their viability assessed using an apoptosis detection kit and with DCF to evaluate the presence of reactive oxygen species (ROS), indicating oxidation levels.

Results: The untreated islets had an average of 95.5% live, 1.1% necrotic and 3.3% apoptotic cells. All islets treated with bayberry extracts showed enhanced viability with the third showing a significant increase in viability (p = 0.03) as well as a significant decrease in the percentage of apoptotic cells (p = 0.01). Of the seven citrus extracts, five showed decrease in islet viability and significant increase in necrosis compared to untreated islets. Two of these, pure naringin, and pure bergamottin had significantly lower viability (p = 0.01 and p = 0.008, respectively). These samples were the only two that exhibited significantly higher percentages of apoptotic cells (p = 0.01, p = 0.02) compared to untreated islets. Qualitative assessment of ROS production showed lower levels of ROS in the three bayberry extracts compared to the untreated control. The five citrus extracts with lower viability showed higher ROS production while the two citrus extracts with higher viability showed similar ROS production to the untreated control. Islets treated with H2O2 and Rapamycin had averages of 37.1% and 52.6% live cells respectively as well as much higher levels of ROS compared to the untreated islets.

Conclusion: Although not all statistically significant, all three bayberry extracts showed higher percentage of islet viability and lower levels of apoptosis. None of the citrus extracts showed significant increases in islet viability.

Keywords: Anti-oxidant, Apoptosis, Necrosis, Islet transplantation, Oxidative stress
TREATMENT OF HUMAN ISLETS WITH CYANIDIN-3-O-GLUCOSIDE LEADS TO AN AUGMENTATION OF AUTOPHAGIC FLUX AND A DECREASED INFLAMMATORY RESPONSE IN VITRO

Jennifer Croden, Kaja Matovinovic, Josué Rodrigues Silva, Gina R. Rayat

Alberta Diabetes Institute, Ray Rajotte Surgical-Medical Research Institute, Department of Surgery, Faculty of Medicine and Dentistry, University of Alberta

Background: Islet transplantation is currently being considered as an alternative treatment for Type I Diabetes. Despite recent progress, transplant patients continue to experience a progressive loss of insulin independence for reasons that are not well understood. Cyanidin-3-O-Glucoside (C3G), an antioxidant found in Chinese bayberry, has been shown to be protective in vitro against the cell damage that may lead to islet loss during transplantation. We hypothesized that C3G is protective because of its ability to regulate autophagy; therefore, human islets treated with C3G should show an increased expression of autophagy marker LC3 and a decreased expression of inflammatory markers NLRP3 and IL-1β.

Methods: Human islets were obtained from the Alberta Diabetes Institute Human Islet Core laboratory and cultured in growth medium with or without C3G for 24 hours. Islets were then treated with human amylin, Aβ1-42, or rapamycin for 24 hours or H2O2 for 2 hours to mimic stresses encountered in the post-transplant environment. Samples of these islets were collected and stained for LC3, NLRP3, or IL-1β. Marker expression was visualized by immunofluorescence microscopy, and quantification of marker expression was performed using ImageJ software.

Results: We found that when islets were treated with stress-inducing human amylin, Aβ1-42, rapamycin, or H2O2, LC3 expression was decreased, whereas expression of NLRP3 and IL-1β was increased. However, when islets were treated with C3G prior to the addition of a stress-inducing agent, LC3 expression was significantly increased (p ≤ 0.01) and NLRP3 and IL-1β expression was significantly decreased (p ≤ 0.01) compared to the samples not treated with C3G. These same findings were seen when this experiment was repeated using a rat INS-1 cell line.

Conclusion: Augmentation of autophagic flux is a likely mechanism by which C3G is protective against cellular stress in vitro. Further examination of the involvement of LC3 using siRNA is needed to confirm the specific mechanism whereby C3G is protective to islet cells.

Key words: human islets, autophagy, C3G, INS-1 cells
REGULAR-FAT CHEESE IMPROVES INSULIN SENSITIVITY IN A PRE-DIABETIC RAT MODEL

Mortaza Fatehi, Xiao-feng Wang, Anik Hanning, Catherine B. Chan

Department of Agriculture, Food and Nutritional Science, University of Alberta

**Background:** Some food guides (e.g., province of Alberta) recommend limiting the amount of regular-fat cheese eaten but this may not reflect emerging scientific evidence of the potential health benefits of regular-fat cheese. This pre-existing notion is based on the belief that a higher fat and saturated fat content affects physical health negatively. However, what this view fails to take into account is that cheese is nutritionally dense and supplies protein and a long list of micronutrients, including calcium, magnesium, zinc, B vitamins, and vitamins A, D and E. Furthermore, certain metabolic effects might be enhanced or blunted, depending on the components and combination of dairy products responsible. We hypothesize that when proteins, lipids, and fatty acids are present in the form and combination as in full fat cheese, they provide a beneficial effect over glucose homeostasis and insulin secretion.

**Methods:** 16 Sprague Dawley rats were acquired at 8 weeks of age and fed normal chow diet for a one week acclimatization period. After which, they were split into four groups: Low Fat Control (LF), High Fat Control (HF: 42% energy from fat -- of which 95% is from lard), High Fat diet + Low Fat-Cheese (HF-LCh), High Fat diet + High Fat-Cheese (HF-HCh). Experimental diets were matched for macronutrients and energy density with fat from cheese replacing fat from lard. The rats were on the diets for nine weeks, after which they were randomized into two groups for Oral Glucose Tolerance Tests (OGTT) and Insulin Tolerance Tests (ITT) testing. Following this, the animals were euthanized and their tissues weighed and collected (including liver, skeletal muscle, adipose tissue, pancreas, jejunum, ileum and ascending colon). Tissue samples were flash-frozen and stored at -80°C. Glucose Stimulated Insulin Secretion (GSIS) was measured in isolated islets with radioimmunoassay.

**Results:** Body weight and food intake were similar between different groups throughout the study, with all groups approaching the average final weights of ~700 grams by nine weeks of experimental diet feeding. No differences in OGTT were detected between HF control and cheese diets. However, both the HF-HCh and HF-LCh group were more responsive to insulin during ITT when compared to the HF control. GSIS results from isolated islets were relatively comparable across all four groups.

**Conclusion:** In a pre-diabetic rat model replacing 20-50% of the fat from lard with cheese improved insulin sensitivity, as measured by ITT. Since both cheese diets elicited a similar effect, despite differences in the fat contribution from cheese, it suggests that a non-fat component of the cheese is responsible for this beneficial effect. Further experiments are required to elucidate the mechanism by which cheese yields an improvement in insulin sensitivity.

**Keywords:** Type 2 Diabetes, Glucose Homeostasis, Insulin secretion/sensitivity, Cheese
ISLET TRANSPLANT PATIENTS DO NOT DISPLAY HYPOGLYCEMIA DURING OR AFTER MODERATE AEROBIC EXERCISE

DEANNA FUNK, SAEED REZA TOGHI ESHGHI, JORDAN REES, CHUFAN ZHANG, BECCA DYCK, NORMAND BOULÉ, PETER SENIOR, KITTY CHEUNG, TOLU OiratejU, JANE YARDLEY

Department of Social Science, Augustana Faculty, University of Alberta

Background: Fear of hypoglycemia is one of the major barriers to exercise in people with type 1 diabetes (T1D). Islet cell transplantation (ITx) has been shown to be effective in restoring insulin independence in this population. Animal studies suggest that glucoregulation may not be completely restored following ITx and that irregular glucose control may still be present. However, the risk of hypoglycemia during and after exercise has not been investigated in human patients.

Methods: Ten insulin-independent ITx individuals performed either 45 minutes of moderate cycling (60% of VO$_{2\text{max}}$) or 45 minutes of seated rest on two separate days. Sessions were assigned randomly and performed at the same time of day (late afternoon), separated by at least 48 hours. Participants were asked to replicate food timing and composition as closely as possible on the two testing days. Mean participant characteristics were as follows: age (50.8 ± 8.7 years), height (165.7 ± 7.4 cm), weight (62.2 ± 9.6 kg), and sex (5 females, 5 males).

Results: Our results show that none of the ITx participants experienced interstitial glucose values (as estimated by continuous glucose monitoring [CGM]) in the hypoglycemic range (<3.9 mmol/L) from the onset of exercise to 12 hours post-exercise. Although CGM glucose values declined during exercise (p<0.001), means from the beginning of exercise to 6 hours post-exercise were not significantly different (p=0.48) between the resting and exercise conditions. However, a significant difference was found (p=0.0024) from 6-12 hours post-exercise with lower means for the exercise condition.

Conclusion: These findings suggest that ITx may restore proper glucoregulation during moderate aerobic exercise in people with T1D. Further research is required to identify blood glucose trends surrounding other exercise modalities, intensities, and durations.

Key words: Islet cell transplantation, type 1 diabetes, exercise
AGE-RELATED DIFFERENCES IN THE REGULATORY CAPACITY OF CD5+CD1D+ B-CELLS IN THE CONTEXT OF HEART GRAFT ACCEPTANCE

Ali Hajar1,2,4; Lavinia Ionescu1,4; Ying Ling1,2; Lori West1,2,3,4; and Simon Urschel,1,2,4
1Pediatrics, University of Alberta, Edmonton, AB, Canada; 2Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada; 3Surgery, University of Alberta, Edmonton, AB, Canada, 4Alberta Institute for Transplant Sciences

Background: Infants benefit from improved graft survival and reduced immunosuppression needs following heart transplantation. The CD5+CD1d+ subpopulation of B-cells contains IL10-producing “B10 cells” found to have immune regulatory capacity in animals. We found this subtype to be ten times more prevalent in infants than adults. CD24+CD38+ (transitional) B-cells were also shown to improve graft survival in animal islet models. We aim to determine if human IL10-producing B-cells are contained in the CD5+CD1d+ or CD24+CD38+ B-cell subsets, and if CD5+CD1d+ B-cells contribute to the development of the more tolerogenic environment in children. Age-related differences in functionality of these cells will be explored.

Methods: Splenocytes were sorted by flow cytometry (FACS) to separate CD5+CD1d+ or CD24+CD38+ B-cells from other B-cells and obtain 4 populations, which were cultured with stimuli inducing Thymus-dependent (TD; IgM+CD40L) or Thymus-independent (TI; CpG) activation. IL10 secretion was measured by ELISA. To assess effects on proliferation, CellTrace™-marked splenocytes were stimulated with Staphylococcal enterotoxin B, IgM+CD40L, CpG, or CD3+CD28 in absence of CD5+CD1d+ B-cells as well as 1(natural proportion, CONTROL), 2 and 5 times their natural proportions.

Results: CD5+CD1d+ B-cells produced IL10 following TD and TI activation. However, TI-activated non-CD5+CD1d+ B-cells produced higher amounts of IL10 than the CD5+CD1d+ subset. In adult samples, IL10 secretion and variation between subsets were greatly reduced, while baseline secretion increased. Compared to the CONTROL, B-cell proliferation after TD activation was higher in CD5+CD1d+ depleted cultures and reduced in samples with 2X the natural proportion. The effects were mainly on memory (CD27+) B-cells. No effects on TI B-cell activation or T-cell proliferation was observed. As with IL10 secretion, CD5+CD1d+ B-cell effects on proliferation were diminished in adult samples.

Conclusion: Many of the regulatory B10 cells are found within the CD5+CD1d+ and CD24+CD38+ B-cell pools, but IL10-producing B-cells with other phenotypes may exist. B10 presence in increasing proportions decreases B-cell proliferation after TD activation. Further analysis is required to confirm age-related difference in IL10 secretion and suppression of proliferation.

Keywords: Regulatory B-cells, CD5+CD1d+ B-cells, Transitional B-cells, CD24+CD38+ B-cells, B10 cells
SLEEP DISORDERED BREATHING IN CHILDREN WITH PRADER-WILLI SYNDROME

Xiao Tian He¹, Michelle Mackenzie¹, Joanna MacLean², Andrea Haqq¹

Division of Endocrinology¹ and Pulmonology², Department of Pediatrics, University of Alberta

Background: Prader-Willi syndrome (PWS) is a disorder characterized by hypotonia, hyperphagia, obesity, growth hormone deficiency and sleep-related breathing disorders. Despite the introduction of growth hormone treatment and improved management of obesity, PWS patients still present with significant sleep disordered breathing (SDB). The etiology for the SDB in PWS remains unclear. Potential mechanisms for their SDB may include hypothalamic dysfunction of circadian rhythm control. The aim of this study was therefore to evaluate the general polysomnographic characteristics of children with PWS.

Methods: This study is a retrospective chart review that evaluated patients who were referred to the pediatric sleep laboratory at the Stollery Children's Hospital over the past 8 years. All patients had a level one overnight polysomnographic study; only the first diagnostic study was included. A total of nineteen PSG studies from nineteen individual PWS patients were selected, including 7 males and 12 females. Data is summarized as median (interquartile range). Age of the patients was 5.5 (2.3 - 9.0) years and BMI z-score was 0.75 (-0.53 - 1.97). Parameters of interest included baseline anthropometrics, sleep efficiency and stage, oxygen saturation, air flow, and respiratory movement.

Results: Overall sleep efficiency was 86.9% (83.3 - 92.7%). Baseline arterial oxygen saturation was 95.4% (93.8% - 97.1%) with a median nadir of 87% (80% - 89%). Oxygen desaturation index (DI) was 2.6 (0.0 - 5.0) with REM oxygen DI of 4.4 (0.0 - 16.9). Apnea index was 2.9 (1.0 - 4.6); hypopnea index was 3.2 (0.8 - 8.2). AHI was 5.7 (4.2 - 12.0); mixed-obstructive apnea-hypopnea index (MOAHI) was 4.3 (0.8 - 8.6) and the central apnea hypopnea index was 1.5 (0.7 - 3.6). Almost all episodes of apnea were of central origin. BMI z-score was negatively correlated with mean SpO2 in REM sleep only ($r_s = -0.457$, $P = 0.049$) and nadir SpO2 in REM sleep ($r_s = -0.0490$, $P = 0.033$). Older age is associated with a higher BMI z-score ($r_s = 0.561$, $P = 0.012$), and a lower total apnea index ($r_s = -0.466$, $P < 0.044$), driven primarily by a decline in obstructive apnea ($r_s = -0.525$, $P = 0.021$).

Conclusion: Consistent with previous studies, sleep hypoxemia and sleep disordered breathing is more prevalent in patients with PWS compared to normal children. Higher BMI z-scores and obesity in PWS children seems to be associated with worse sleep hypoxemia, particularly in REM sleep. With increasing age, the profile of apnea is less obstructive in nature. Further studies with BMI-z-matched control subjects are required to delineate how age, weight status, and the syndrome itself contribute to the progression of SDB in children with PWS. Ultimately, targeted treatment of sleep abnormalities in patients with PWS will help to optimize their daily functioning, learning, metabolic health and quality of life.

Key words: Prader-Willi syndrome, sleep-disordered breathing, polysomnographic study
**Secretory Change of Cryopreserved Human Islets After Culture Under Maturation Conditions**

Ryekjang Kim, Aliya Spigelman, Karen Seeberger, James Lyon, Jocelyn E. Manning Fox, Gregory Korbutt, Patrick E. MacDonald

Department of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta

**Background:** Cryopreservation of islets can potentially address shortcomings of clinical islet transplantation such as donor availability. However, we have previously observed abnormal insulin secretion in these cryopreserved cells. The underlying process causing the decrease in islet function is of interest and beta-cell de-differentiation is one possible explanation, particularly since cryopreserved islets have similar secretory profiles to infant islets.

**Methods:** Isolated islets from six deceased donors without diabetes were cryopreserved for 326 ± 97 days. Islet function was measured by glucose-stimulated insulin secretion and β-cell exocytosis by whole cell patch clamp following isolation (freshly isolated), immediately after thawing (cryopreserved), after two weeks of standard culture (cultured), or after a two-week long, step-wise maturation protocol of Oncostatin M, dexamethasone, nicotinamide, exedin-4, transforming growth factor beta-1, and thrombin (maturation cultured).

**Results:** The control islets from two of the first three donors did not survive the two-week culture after thawing, and as a result we compared the maturation cultured islet responses with cryopreserved and freshly isolated responses. Insulin secretory responses were not significantly different between freshly isolated islets, cryopreserved islets, and following maturation culture. However, insulin content decreased significantly, and relative insulin release during low glucose incubation seemed higher in both cryopreservated and maturation cultured islets compared with freshly isolated islets. β-cells from cryopreserved islets had significantly lower exocytotic responses compared to that of freshly isolated controls, which did not change after maturation culture. Surprisingly, the maturation cultured β-cells had marginally higher exocytosis in response to 1 mM than to 10 mM glucose. In comparison, post-cryopreservation β-cells exhibited no such difference, and β-cells from freshly isolated islets had higher exocytosis in 10 mM glucose than in 1 mM glucose as expected.

**Conclusion:** Although the maturation protocol promoted islet survival, β-cell exocytosis was not restored to pre-cryopreservation levels. As well, both patch clamp and glucose stimulated insulin secretion measurements indicate that the islets seem to lose proper glucose sensitivity upon cryopreservation that is not restored by maturation treatment.

**Key Words:** Cryopreservation, Islets, Secretion, Exocytosis, Maturation
LYMPHOCYTE ALTERATIONS IN HEART-TRANSPLANTED CHILDREN IN RELATION TO DEVELOPMENT OF ALLERGIC AND AUTOIMMUNE DISORDERS

Tiffany B. Kim; Lavinia Ionescu, Nicholas Avdimiretz, Lori J. West, Simon Urschel

Departments of Pediatrics and Medical Microbiology & Immunology, University of Alberta

Introduction: Pediatric heart-transplant recipients are at higher risk for allergic and autoimmune disorders. We previously found a young age at transplantation and thymectomy to be associated factors. We hypothesized that thymectomy and immunosuppressive therapies at an early age affect the development of T- and B-cell subsets, especially Tregs, which are important in maintaining peripheral tolerance.

Aim: To investigate the impact of thymectomy and lymphocyte depleting induction therapy on lymphocyte subtype proportions in relation to an increased risk of asthma and allergies.

Methods: The presence and proportions of lymphocyte subpopulations (B-cell, T-cell, Breg, Treg) were determined by flow cytometry phenotyping. The expression of specific surface markers was examined. Clinical data were collected in standardized questionnaires examining allergic and autoimmune disorders including asthma and eczema, as well as severity, time of development, and treatment. Medical charts were reviewed for data consolidation.

Results: The proportion of CD45RA+CD27+ naïve, Treg cells within the complete Treg population, was found to be lower in thymectomized patients than non-thymectomized ones. Patients with the lowest proportions of this subset developed asthma post-transplant. However, this correlation remains observational due to the small sample size. Memory CD4+ cell proportions were higher in thymectomized patients as well. No consistent trends were identified when examining Breg and transitional B-cell populations.

Conclusions: Lower percentages of certain Treg subsets, following thymectomy, within the lymphocyte population, may lead to an increased risk for the development of disorders such as asthma. Memory, regulatory and transitional B and other lymphocyte subsets may also contribute to this increased vulnerability, although this was not shown in our small sample. A larger sample size will allow us to identify whether clear correlations exist correlation to the clinical findings.

Key Words: Thymectomy, induction, asthma, tregs
BIOPRINTING HIGH CELL DENSITY AND VASCULARIZED TISSUES

Douglas Kondro, Derek Toms and Mark Ungrin

Department of Mechanical and Manufacturing Engineering, Faculty of Engineering, U of C.

**Background:** A major limitation of islet cell transplantation is the inability to replicate physiological tissue size due to the lack of vascularization. One possible approach of increasing the complexity of implanted islets is to utilize bioprinting but in order to mitigate cell loss and density problems biomaterials can be used as a feedstock. The biomaterials can be used to form complex scaffolds and molds for cell constructs. Miller et al (Nature Materials, 2012) published an innovative approach to the vascularization of engineered tissues employing a sacrificial carbohydrate glass template, coated with the polymer poly(d-lactide-co-glycolide). A cell construct composed of a single cell suspension and aqueous-based extra cellular matrix can be cast over the carbohydrate glass template and the carbohydrate structure can be dissolved away to produce a vascular network. This approach has been limited in reaching physiological cell density. I will present ongoing work on a new approach that aims to overcome this challenge by investigating different methods of incorporating high density cell structures into the vascularized lattice structures.

**Methods:** The carbohydrate glass template was produced with a mixture of 18% glucose, 38% sucrose, 7.2% dextran and 36.2% osmotic water. The mixture was warmed up to 165°C to form a glass and then the mixture was placed into a 50 mL metal syringe. A temperature of 125°C and 65 psi was utilized for printing. 250 mg of PCL was dissolved in 10 mL of chloroform to create the polymer coating. A 2%-4% agarose and media hydrogel was created with a H9 cell line suspended into the agarose.

**Results:** A vascular network has been created with a cell suspension of in 3% agarose. The structure experienced cell death after three days however cell concentration was increased from the single cell suspension. Further testing is ongoing.

**Conclusion:** Methods of increasing the cell density of 3D printed vascularized tissue was investigated. Although testing is ongoing this method has produced a possible method of developing vascular networks that may be implemented in islet transplantation to improve their survival rate.

**Key Words:** Bioprinting, vascularization, islet transplantation
THE IMPACT OF VASCULAR ENDOTHELIAL GROWTH FACTOR ON ANGIOGENESIS IN A SUBCUTANEOUS ISLET TRANSPLANT SITE

Purich, K., Pepper, A., Bruni, A., Gamble, A., Pawlick, R., Kuppan, P., Korbutt, G., Shapiro, J.
Shapiro Laboratory, Alberta Diabetes Institute, Department of Surgery, University of Alberta Faculty of Medicine. Funding provided by Alberta Diabetes Institute

**Background:** Islet transplantation has become an attractive treatment option for type 1 diabetics. However, the current transplant method of portal vein infusion poses risks, including: portal vein thrombosis, bleeding and the inability to retrieve a graft after transplantation. The Shapiro Laboratory has recently had success through the use of a subcutaneous transplant site, which reduces transplant risk and allows for safe graft retrieval. Prior to the subcutaneous islet infusion, the transplant site must be vascularized, a challenge that has been overcome by temporary pre-transplant implantation of a nylon angiographic catheter. In our study, we implant functionalized catheters sporting different properties, attempting to enhance angiogenesis within the subcutaneous site. In theory, these improvements should maximize the amount of blood flow to the transplant site, improving islet survival, and subsequently improving subcutaneous islet transplant effectiveness.

**Methods:** We implanted 1 cm long, 6 French diameter subcutaneous catheters into 12 BALB/c mice. Each mouse had three catheters sporting different properties placed under the skin on the ventral abdomen, with approximately 1 cm of separation in between the catheters. A nylon catheter was used as an internal control and the two experimental groups consisted of the same nylon catheter coated with a biodegradable polymer scaffold known as poly-epsilon-caprolactone (PCL), as well as a nylon catheter coated in PCL impregnated with a Vascular Endothelial Growth Factor (VEGF) synthetic mimic. Grafts were removed at 1, 2, 3 and 4 weeks. Angiogenesis was quantified through the use of Von Willebrand factor antibody staining. Anticipating positive results, we have implanted single 2 cm catheters into ten C57BL/6 mice, separating them into a treatment group in which the mice have a VEGF impregnated PCL coated nylon catheter implanted, and a control group in which the mice have a non-coated nylon catheter implanted. We plan to remove the catheters and complete a subcutaneous murine islet transplant on these mice. Afterwards we will monitor blood glucose levels and look for significant differences between the treatment and control group in regards to islet graft effectiveness.

**Results:** We anticipate an increased amount of angiogenesis to be seen in the mice with the VEGF treated catheters in comparison to our control, and no significant difference between the non-VEGF impregnated PCL coated catheter and our control. In addition, we expect that the VEGF treatment group will become euglycemic after islet transplant at an earlier timepoint, and have a greater proportion of mice that become euglycemic in comparison to the controls.

**Conclusion:** We anticipate that VEGF embedded scaffolds will promote greater levels of blood vessel growth within the subcutaneous transplant site, improving islet transplant effectiveness.

**Keywords:** Subcutaneous islet transplantation, VEGF, Angiogenesis
IN VITRO EVALUATION OF THE OPTIMAL IMMUNOSUPPRESSIVE RATIO OF TREGS TO ISLETS

Yasmin Rafiei, Andrew Pepper, Rena Pawlick, Antonio Bruni, Esme Dijke, Anne Halpin, Morgan Sosniuk, A.M. James Shapiro

Department of Surgery, Faculty of Medicine and Dentistry, University of Alberta

Background: Human thymuses, routinely removed during pediatric cardiac surgeries, are a novel source of stable and high yield CD4+CD25+FOXP3+ T regulatory cells (Tregs) demonstrating therapeutic potential and thus, provide an attractive co-transplant option for islet allotransplant. In murine transplantation models, islets co-transplanted with Tregs under the kidney capsule have exhibited prolonged in vivo graft function (Krzystyniak et al., 2014); however, this remains clinically unfeasible due to potential kidney damage. In order to capitalize on their immuno-protective potency, it has been postulated that Tregs should be co-localized with islets. Consequently, the goal of this project was to establish the suppressive capacity of Tregs on islet-induced T cell proliferation with the aim of determining the optimal ratio of islets to Treg cells to yield an immunomodulatory effect in a subcutaneously transplanted murine model.

Methods: To determine the optimal ratio of islets to Tregs, a mixed lymphocyte reaction (MLR) was employed using human islets as stimulators and normal, healthy human peripheral blood mononuclear cells (PBMCs) as responders. Expanded thymic Tregs were incorporated in subsequent MLRs to characterize their optimal immunosuppressive ability. Human research islets, acquired from the Clinical Islet Laboratory, Edmonton, Alberta, were washed and irradiated at 40 Gray, while PBMCs were isolated from whole blood via Ficoll gradient. Islets were plated at 25, 50, 75, and 100 IE/well against PBMCs at 5x10^4, 1x10^5, or 5x10^5 cells/well. Thymic Tregs were subsequently added at 2.5x10^3, 5x10^3, 1x10^4, and 2x10^4 cells/well. Four days post-culture, culture media was replenished and cell-free supernatants harvested for future cytokine analysis. Seven days post-culture, cell-free supernatants were harvested again and proliferative responses were analyzed using flow cytometry by CFSE assay while co-labeling CD3, CD4, and CD8 cell markers.

Results: MLRs with islets plated against PBMCs yielded a proliferative response of CD3 T-cells: the cell population was highly characterized by CD4 T-cells with low to moderate representation from CD8 T-cells. Subsequent experimentation with Tregs indicated suppressive capacity.

Conclusions: Co-culture of islets with Tregs demonstrates promise as a future immunomodulatory treatment for in vivo experimentation. Transplantation of islets and Tregs, sub-kidney capsule and subcutaneously in the murine model, pose an attractive option for future research in evaluating the potency of Tregs as a method of improving graft survival and diminishing rejection rates post-transplant.

Key words: Regulatory T-cell, Mixed Lymphocyte Reaction, Islets, Immunomodulation, Co-culture
CYANIDIN-3-O-GLUCOSIDE (C3G) IMPROVES THE VIABILITY AND FUNCTION OF HUMAN ISLET CELLS TREATED WITH HUMAN AMYLIN OR Aβ1-42 IN VITRO

Josue Rodrigues Silva1, Wen Fu2, Jack Jhamandas2, Gina Rayat1.

Alberta Diabetes Institute, 1Department of Surgery and 2Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta

Background: Amyloid deposition is linked to different diseases such as type 2 diabetes and Alzheimer's disease in which amyloid is formed by islet amyloid polypeptide (IAPP, also called amylin) and amyloid β (Aβ), respectively. Amyloid aggregation has been thought to induce processes that lead to a perturbation in the structure of lipid membranes, which subsequently disturb cellular ion homeostasis and trigger a cascade of events, including the formation of reactive oxygen species (ROS). Flavonoids are known to have antioxidant property and are capable of protecting islets from the harmful effect of ROS. Previously we showed that C3G a flavonoid found in Chinese bayberry (Myrica rubra) enhanced the function of mouse and pig islets after transplantation into diabetic mice. In this study, we tested the effect of C3G on human islets in vitro.

Methods: Human islets were cultured with or without C3G for 24 hours, and then treated with human amylin or Aβ1-42 at physiological conditions. Samples were stained with Thioflavin-S, Calcein/Ethidium and dichlorodihydrofluorescein diacetate to evaluate the amyloid aggregation, cell viability and ROS by ImageJ and stereological analysis. The ultrastructure of islets was also assessed using transmission electron microscope (TEM). The in vitro function of human islets was measured using static glucose stimulation assay where islets were exposed to low glucose [2.8mM] and high glucose [20mM] concentrations following our standard protocol. The amount of insulin produced during incubation with glucose was determined using MSD insulin kit.

Results: Treatment of human islets with C3G reduced the amyloid-like deposition compared to untreated islets. C3G treatment also improved the survival of islet cells and reduced the presence of ROS in the islets that were exposed to human amylin or Aβ1-42. TEM images of islet cells treated with C3G showed an increased autophagic activity in contrast with other conditions. In addition, when C3G treated islets were incubated with 20mM glucose higher amount of insulin was detected compared to untreated islets. Similar results were found in human islets treated with C3G plus hAmylin or Aβ1-42 compared with islets treated with only hAmylin or Aβ1-42.

Conclusion: Our preliminary results indicate that C3G could enhance the survival and function of human islets in vitro by reducing amyloid-like aggregates and ROS and by increasing autophagy as a survival mechanism under oxidative stress.

Keywords: human islets, C3G, amyloid, ROS, viability
OLD DOGS WITH NEW TRICKS: OPTIMIZING MIXED LYMPHOCYTE REACTION WITH DURACLONE FLOW CYTOMETRY PHENOTYPING

Morgan Sosniuk¹, Anne Halpin¹, Simon Urschel¹, Patricia Campbell², Lori West¹, Anne Halpin¹, Simon Urschel¹, Patricia Campbell², Lori West¹
¹Department of Pediatrics, University of Alberta (U of A), Edmonton, AB, ²Alberta Transplant Institute, Edmonton, AB

BACKGROUND: Cardiac transplantation is a lifesaving intervention but despite immunosuppression, the immune system may damage the transplanted organ via responses such as antibody-mediated rejection. In pediatric cardiac surgeries thymectomy is routinely performed. Our overarching goal is to study the impact of thymectomy and its role in the development of de novo donor-specific Ab (DSA) in this population. The Beckman Coulter Duraclone IM flow cytometry phenotyping reagents are widely used within the Canadian National Transplant Research Program (CNTRP) and provide standardized data from centre to centre. Here, we investigate this method incorporated with mixed lymphocyte reaction (MLR) analysis of pediatric post-transplant peripheral blood mononuclear cells (PBMCs).

METHODS: Pre- and post-transplant HLA antibody data were pulled from the clinical HLA laboratory database and analysed. MLR and flow phenotyping were optimized using PBMCs and irradiated pooled third-party splenocytes. Proliferation was quantified using CellTrace Violet proliferation dye combined with Duraclone IM (DuraFlow) phenotyping or BrdU incorporation ELISA (n=10). These methods are conducive to similar analysis and experimentation with alternate stimulator cells, such as islet cells.

RESULTS: There were 151 patients, 118 of whom had pre- and post-transplant HLA antibody testing (results not shown). The BrdU assay demonstrated proliferation but lacks detail regarding individual cell population responses. The proliferation dye was readily detected within the DuraFlow panel. Unstimulated cells did not proliferate whereas PHA stimulated cells showed abundant proliferation. There were clear phenotypic changes from pre- to post-MLR including the disappearance of monocytes and decreased NK populations. T and B cell populations were clearly labelled and well-defined in the flow analysis. Comparable percentages of cell populations were detected in DuraFlow tubes using $0.2 \times 10^6$ cells or $1.0 \times 10^6$ cells.

CONCLUSION: Our preliminary results show that Duraclone IM is a useful system for detecting proliferation responses in MLR combined with phenotypic analysis of cultured cells. These results suggest that DuraFlow can be used not only as a standardised flow phenotyping method but has a novel application in the setting of MLR. This provides a unique opportunity to exploit the DuraFlow tools in the investigation of immune responses. Furthermore, the processes used to extract and analyse the HLA antibody data for this heart patient population could be efficiently reproduced for the islet transplant population to explore HLA sensitization in these patients.

KEYWORDS Alloimmunization, HLA, MLR, flow cytometry phenotyping
BLOOD GROUP A-ANTIGEN-SPECIFIC TOLERANCE FOLLOWING INFANT A-ANTIGEN EXPOSURE IN A MOUSE MODEL OF ABO-INCOMPATIBLE HEART TRANSPLANTATION (ABOi HTx)

Yiqun Wang, Brendon Lamarche, Ibrahim Adam, Jean Pearcey, Kesheng Tao, Chris W. Cairo, Bruce Motyka, and Lori J. West

Department of Pediatrics, Faculty of Medicine and Dentistry, University of Alberta

Background: Life-saving ABOi HTx can be performed safely in infants as a result of absent-low levels of anti-A/B antibody (Ab). Tolerance develops to donor blood group A/B-antigen(s) following ABOi HTx by mechanisms not well understood. To further study ABO-related transplant immunobiology, we generated an A-transgenic (A-Tg, C57BL/6 (B6) background) mouse. We showed A-antigen specific tolerance in this model following Tx of A-Tg hearts into young (4 week-old) wild-type B6 (WT) mice. Herein, we sought to explore induction of A-antigen-specific tolerance with administration of A-antigen in a form other than a graft. A-antigen is present at high levels in A-Tg blood cells. We hypothesize that A-antigen-specific tolerance would be induced following treatment of infant WT mice (≤3 weeks of age) with A-antigen expressing A-Tg blood cells (RBCs).

Methods: WT mice at 7, 14, and 21 days of age were injected intraperitoneally (i.p.) with: 1) intact A-Tg RBCs (n=12); 2) A-Tg RBC membranes (n=4); 3) human A RBC membranes (hA-RBCs) (n=6); or 4) left untreated (n=9). As adults (7 weeks), all mice were injected i.p. with hA-RBCs (‘A-sensitized’) in an attempt to elicit anti-A Ab production. Serum anti-A and 3rd-party (anti-human RBC) Ab were assessed by hemagglutination assay.

Results: Following A-sensitization, high levels of anti-A Ab developed in mice untreated as infants (median titre 1:1024), treated with hA-RBC (median titre 1:2048) or ATg RBC membranes (median titre 1:256). In contrast, anti-A Ab remained undetectable-low in A-sensitized mice treated as infants with intact ATg RBC (median titre ≤1:2); 3rd-party anti-human RBC Ab levels were high (median titre 1:64). Intact A-Tg RBC (mean 2.5 ng/mL) and A-Tg RBC membrane (undetectable) treated groups had less IgG anti-A Ab compared to hA-RBC treated (mean 45 ng/mL) and untreated groups (mean 15 ng/mL).

Conclusions: The ability to elicit 3rd-party but not A-antigen-specific Ab in A-sensitized mice treated as infants with intact A-Tg RBCs suggests the development of robust A-antigen-specific tolerance. Future studies will investigate A-antigen persistence following infant injection and use of A-antigen-expressing glycoconjugates for tolerance induction.

Keywords: ABO-incompatible, Heart Transplantation, Tolerance
PREGNANT WOMEN WITH DIABETES MELLITUS IMPROVE THEIR DIET BY REDUCING SUGAR INTAKE.

Hillary A. Wilson, Ye Shen, Caroline Richard, Amy Weinberg, Brenda Leung, Rhonda C. Bell, Catherine J. Field and the APrON team, Dept of AFNS, University of Alberta

INTRODUCTION
Pregnant women in Alberta who meet the criteria for diabetes mellitus (DM) receive nutritional counseling. Recently, high intake of energy and simple sugars (specifically fructose) has been associated with an increased risk of negative maternal and infant outcomes. It is not known if energy and carbohydrate content of diets of women with DM differs from those without DM. We compared the dietary intake of women participating in the Alberta Pregnancy Outcomes and Nutrition (APrON) cohort who were diagnosed with DM relative to those without DM.

METHODS
Using the APrON cohort, 115 women were identified as having DM using hospital records or a questionnaire. Nutrient intake was assessed from 24-hour dietary intake recalls collected during each trimester, beginning with the 1st or 2nd trimester depending on date of enrolment and one at 3 months post partum. Food Processor was used to determine the nutritive value of foods recalled. Intakes of women with DM were compared to the larger cohort (n=1843).

RESULTS
Energy and fiber intake did not differ between women with DM and the full cohort. However, in the 3rd trimester, DM women reported a lower proportion of energy from carbohydrates (49 ± 9% vs. 54 ± 9, P<0.05) and total sugar (17 ± 7% of energy vs. 21 ± 8% P<0.05) and less than that reported in the Canadian Community Health Survey for non-pregnant women (20% of energy). This was compensated by a higher proportion of calories from protein and fat compared to the APrON cohort. Dietary calcium intake was also higher in DM women (1310 ± 659 mg/d vs.1159 ± 547mg/d, P<0.05), suggesting the higher protein and fat may have come from dairy foods. At 3 months post partum, the proportion of energy from carbohydrates remained significantly lower in DM women (P<0.05). For women with DM, energy intake was similar to the 1st trimester but the proportion of energy from carbohydrates was lower and remained lower than the APrON cohort (P<0.05). Sugar intake (20 ± 11% vs.17 ± 11% of energy) did not differ between groups.

CONCLUSIONS
Women with DM during pregnancy made positive changes in their diet, by reducing simple sugar and carbohydrate intake and consuming more calcium containing foods. Sugar consumption was higher in all women than Health Canada and Canadian Diabetes Association recommends. Our results suggest that all women should receive advice during pregnancy so as to meet current recommendations for sugar intake.

Key words: nutrition, diabetes mellitus, carbohydrate intake, pregnancy
CHARACTERISTICS OF VITAMIN D SUPPLEMENT USERS VS NON SUPPLEMENT USERS IN AN AMBULATORY POPULATION WITH DIABETES AND CHRONIC KIDNEY DISEASE (CKD).

Adame Perez S, Seto L, Jindal K, Senior P, Mager DR

Department of Agricultural, Food and Nutritional Science, Endocrinology, Nephrology, and Pediatrics, University of Alberta; Diabetic Nephrology Prevention Clinic and Northern Alberta Renal Program, Alberta Health Services.

**Background.** Vitamin D insufficiency is highly prevalent in adults with diabetes mellitus (DM) and chronic kidney disease (CKD), particularly in northern communities due to dietary restriction of vitamin D rich foods and reduced sunlight exposure. While many patients are prescribed vitamin D by health care practitioners, adherence to supplementation can vary substantially within this population. The study purpose was to describe the differences in anthropometric, demographic and quality of life factors between adults taking multi-vitamins and/or single preparations of vitamin D in an adult population with DM and CKD.

**Methods.** A prospective, ongoing longitudinal study in adult patients with DM/CKD examining factors influencing vitamin D status, bone health and quality of life (QoL) is currently in progress. Variables assessed in this interim analysis include: demographic (age, gender, DM type/duration, CKD stage, insulin use, medication and co-morbidity count), anthropometric (weight, height, BMI), vitamin D status (25(OH)D3), and QoL (SF-36).

**Results.** Mean (±SD) age of study participants was 66.8 ± 8.2 years. A total of 10, and 15 participants were taking either single vitamin D preparations or a combination of single vitamin D preparations and vitamin D containing multi-vitamin, respectively. No differences in age, gender, DM type/duration, CKD stage (1-2 vs 3-4), co-morbidity count or medication numbers were noted between multivitamin/single preparation users vs non-users. Multivitamin/single preparation use was associated with higher serum 25(OH)D (p<.001), increased Mental Health (MH) (p=0.01), and Mental Component Summary (MCS) scores (55.7 ± 7.7 users vs. 51.1 ± 11.3 non users; p= 0.04) in users vs non-users.

**Conclusions:** Multivitamin supplements/single preparations containing vitamin D was associated with increased QoL scores related to mental health, but not to demographic, anthropometric, co-morbid burden or total medication use in an ambulatory population of DM/CKD. Implications for clinical practice will be discussed.

**Key Words:** Diabetes, vitamin D, kidney, multivitamin.
MITOCHONDRIAL DYSFUNCTION PRECEDES DIABETIC RETINOPATHY

Ted Han, Jillian Schneider, Helene Lemieux, Yves Sauve

Department of Physiology

**Background:** Previous studies in rodent models of type 2 diabetes have reported defects in the mitochondrial electron system in various organs, including the eye. With its early onset, mitochondrial dysfunction is a potential outcome measure when developing preventative therapies for diabetic retinopathy; but, the role of mitochondrial dysfunction in relation to the mechanistic onset and progression of the disease remains largely elusive. The present study aims to provide a detailed temporal and mechanistic account of functional changes in mitochondrial respiration complexes. We hypothesise that mitochondrial dysfunction in the retina will precede the onset of diabetic retinopathy.

**Method:** Two diet groups of Nile rats were studied at 2, 6, and 18 months age groups, fed standard rodent chow diet (*Prolab* 2000) or low energy diet (*Mazuri Chinchilla*). Body weight, length, and fasting glucose (FBG) were measured prior to euthanasia. Eyes were then collected and the retina and “eyecup” (containing retinal pigment epithelium (RPE) cells) were isolated. Substrate-uncoupler-inhibitor injections with OROBOROS Oxygraph-2k high-resolution respirometry allowed analysis and quantification of the functional status of individual components of the mitochondrial respiratory system, as well as the integrity of mitochondrial membranes.

**Result:** At 2 months, Nile rats were normoglycemic (<5.0 mmol/L) in both groups with 3.6 ± 2.2 mmol/L (n = 17); but Prolab males were hyperglycemic at 6 months and 18 months, with mean FBG of 11.6 ± 9.7 mmol/L (n=5) vs. Mazuri's 3.3 ± 0.3 mmol/L (n=8; P<0.001), and 11.1 ± 7.6 mmol/L (n=5) vs. 3.3 ± 1.0 mmol/L (n=4; p<0.05) for 6 and 18 months, respectively. Age- and sex-matched diet groups showed no differences in mitochondrial Complex I LEAK respiration throughout retina and eyecup tissues of 3 age groups. Complex I OXPHOS capacity of the retina from 18 month Mazuri animals were higher than corresponding Prolab animals (0.623 ± 0.013, n=4 vs. 0.583 ± 0.015, n=6; P<0.05), whereas retina and eyecup of the remaining age groups did not show any differences. Complex II OXPHOS capacities only showed differences at 18 month male animals' retina (Mazuri 0.495 ± 0.013, n=4 vs. Prolab 0.537 ± 0.023, n=6; P<0.05). Complex IV activities of eyecup mitochondria were higher in the Prolab males compared to corresponding the Mazuri at 6 months (4.140 ± 1.260, n=5, vs. 2.789 ± 0.728, n=7), and at 18 months (2.985 ± 1.049, n=6, vs. 1.643 ± 0.157, n=3). Retina of Prolab males at 2 months, showed higher cytochrome C effect than Mazuri counterparts (0.994 ± 0.014, n=5 for Prolab vs. 0.961 ± 0.022, n=8 Mazuri; P<0.05), while the remaining groups showed no difference.

**Conclusion:** A quantifiable change in retinal mitochondrial respiration precedes onset of hyperglycemia in the Nile rat model of type 2 diabetes. In addition, mitochondrial respiratory complexes of both retina and RPE are differentially affected by hyperglycemia.

**Keyword:** Mitochondria, Diabetic Retinopathy, Type 2 Diabetes
BODY COMPOSITION, HANDGRIP STRENGTH, PHYSICAL CAPACITY AND MARKERS OF CARDIOMETABOLIC AND LIVER DYSFUNCTION IN CHILDREN WITH NONALCOHOLIC FATTY LIVER DISEASE AND PRADER-WILLI SYNDROME.

MacDonald K, Haqq AM, Yap J, Mager D.R.
Department of Agricultural, Food and Nutritional Science, University of Alberta, Department of Pediatrics, University of Alberta,

**Background:** The study purpose was to describe and contrast body composition and markers of muscle strength, physical capacity, cardio-metabolic and liver dysfunction in obese children with nonalcoholic fatty liver disease (NAFLD) and Prader-Willi Syndrome (PWS).

**Methods:** Children aged 7-18 years with NAFLD (n=6), PWS (n=8) and healthy lean controls (n=14) were recruited from the Stollery Children’s Hospital and the community. Anthropometrics (weight, height, circumferences, skinfolds), body composition (DXA), handgrip (measure of muscle strength), 6 minute walk test (6MWT), cardiometabolic and biochemical (blood pressure, triglyceride (TG), total-cholesterol (TC), HDL-and-LDL-cholesterol, glucose, insulin) measures were assessed. Insulin resistance was assessed using HOMA-IR (abnormal > 3).

**Results:** Mean age of the cohort was 12.6 ± 3.4 years, with no differences between groups (p>0.05). NAFLD and PWS children had higher weight-z, BMI-z, waist-to-height-z ratio, waist-circumference-z, insulin and HOMA-IR values compared to healthy controls. However, NAFLD children had significantly higher WC-z, BMI-z, bicep skinfolds and fasting concentrations of ALT when compared to PWS children (p<0.05). There was also a non-significant trend (p=0.09) for PWS children having lower insulin levels compared to NAFLD. No other differences in biochemical (TG, HDL, LDL, TC, glucose, HOMA-IR) measures were observed between NAFLD and PWS, although NAFLD children had significantly higher values for TG, LDL, TC and lower values for HDL than controls. Body Fat% was 43.3% and 44.9% in NAFLD and PWS children, respectively (p>0.05). Children with PWS had significantly lower handgrip strength compared to NAFLD and control children (p=0.04). There was a positive correlation between fat free mass and handgrip strength (p<0.01) for all groups. NAFLD and PWS children had a significantly lower total distance walked in the 6MWT than controls (p<0.05), but no differences between PWS/NAFLD children were observed (p>0.05).

**Conclusion:** Obese children with NAFLD/PWS experienced similar derangements in cardio-metabolic markers, body composition and physical capacity when compared to lean children. However, significant unique reductions in markers of muscle strength were observed in the children with PWS. Further investigations elucidating the potential mechanism/lifestyle factors influencing these findings are warranted.

**Keywords:** Pediatric Obesity, Non-alcoholic fatty liver disease, Prader-Willi Syndrome
INHIBITION OF PHOSPHATIDYLETHANOLAMINE N-METHYLTRANSFERASE WITH ANTI-SENSE OLIGONUCLEOTIDES

Sereana Wan, Jelske N. van der Veen, Mark Graham, René L. Jacobs and Dennis E. Vance

Department of Biochemistry, Faculty of Medicine and Dentistry, University of Alberta

Background: Phosphatidylethanolamine N-Methyltransferase (PEMT) is a membrane protein that catalyzes ~30% of hepatic phosphatidylcholine biosynthesis. When Pemt⁻/⁻ mice are fed a chow diet, they are outwardly indistinguishable from their wild type counterparts. However, when Pemt⁻/⁻ mice are fed a high fat diet (HFD), they are protected from diet induced obesity and insulin resistance, but develop steatohepatitis. We hypothesized that the knock-down of PEMT in wild type mice would confer protection against diet induced obesity and insulin resistance.

Methods: Antisense oligonucleotides (ASO) were designed to inhibit PEMT. Wild type mice were fed the HFD for 10 weeks with weekly administrations of either a scrambled control ASO or an ASO to PEMT. Glucose and insulin tolerance tests were conducted after, respectively, 6 and 7 weeks of HFD. PEMT activity and hepatic TG levels were measured from fresh liver homogenates.

Results: Hepatic PEMT activity was inhibited by 90-100 percent by the ASO-PEMT. Mice treated with the ASO-PEMT gained significantly less weight than the control group and had lower fasting blood glucose levels. The ASO-PEMT-treated mice were significantly more sensitive to both glucose and insulin administration compared to control mice. However, administration of ASO-PEMT also caused development of hepatic steatosis.

Conclusions: Knocking down PEMT in wild type mice confers protection against diet induced obesity and insulin resistance. Future dose studies with ASO-PEMT will be conducted to achieve an optimal range to confer the beneficial effects without development of steatosis. (Research supported by Grant MOP-5182 from the Canadian Institutes of Health Research.)

Key Words: PEMT knock down, ASO, insulin resistance, diet induced obesity
STATE- AND USE-DEPENDENT BINDING OF KCNQ CHANNEL OPENERS


Department of Pharmacology, Faculty of Graduate Studies and Research, University of Alberta

**Background:** Voltage-gated potassium channel openers are an emerging therapeutic modality, currently used for treatment of epilepsy, but with possible applications in pain, vascular disease and others. The development of this class of compounds may contribute to the treatment of the many vascular and neurological complications of diabetes. Retigabine, a KCNQ2-5 channel opener, is the first and only voltage-gated potassium channel opener approved for human use. An important unexplored feature of retigabine and its derivatives is their state- and use-dependent properties. As described for voltage-gated sodium channel blockers, drugs that exhibit use-dependence may have a stronger drug effect with more frequent channel stimulation, making them especially useful for selectively targeting hyperactive cells.

We aimed to generate a detailed understanding of the mechanism of action of KCNQ channel openers. We investigated the state- and use-dependence of interaction of retigabine and a `second-generation' KCNQ opener (ICA069673) with WT KCNQ2 and KCNQ2[A181P] channels.

**Methods:** We used patch clamp electrophysiology recordings of heterologously expressed KCNQ potassium channels, along with rapid solution exchange approaches to investigate channel interactions with various KCNQ openers. We assessed drug binding to pre-open states by applying drugs at different holding potentials, and subjected cells to frequent repetitive stimulations to assess use-dependent drug interactions.

**Results:** WT KCNQ2 channels showed marked state- and use-dependent binding to ICA73, with the drug preferentially binding to the open channel state and having a greater effect with more frequent pulses. Unlike WTQ2, A181P appears to lack state-dependent binding to ICA73, and does not show use-dependent activation. Retigabine binds to both the open and closed WT KCNQ2 channel states, although it may have a higher affinity for the open state, allowing it to retain some use-dependent activation.

**Conclusion:** Our findings highlight use-dependent properties of certain KCNQ channel openers and will contribute to the understanding and enhancement of these beneficial pharmacological features.

**Key words:** KCNQ channels, KCNQ channel openers, use-dependence, epilepsy, electrophysiology
DOES DIETARY CHOLINE OR TMAO SUPPLEMENTATION INCREASE ATHEROSCLEROSIS IN Ldlr−/− MICE?

Paulina Aldana-Hernandez1, Yumna Zia1, John P Kennelly1, Kelly-Ann Leonard1, Si Mi1, Catherine Field1, Jonathan Curtis1, Rene Jacobs1,2 1Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton; 2Department of Biochemistry, University of Alberta, Edmonton

Background: The essential nutrient choline plays an important role in cell membranes formation, lipoprotein secretion and methyl-group metabolism. Recently, choline and TMAO supplementation have shown to enhance atherosclerosis in ApoE−/− mice. It has been proposed that choline is converted to trimethylamine (TMA) by gut microbiota; TMA is then oxidized to trimethylamine N-oxide (TMAO) by the liver enzyme, flavin-containing monoxygenase-3. Furthermore, circulating choline, betaine (the oxidized form of choline) and TMAO have been associated with increased risk of CVD in numerous human trials. The aim of this study was to determine whether dietary choline, betaine or TMAO promote atherosclerosis in low-density lipoprotein receptor knockout (Ldlr−/−) mice.

Methods: Ldlr−/− male mice (N=17 and N=15), aged 8-10 weeks, were fed with high-fat diet (40% of calories and 0.5% of cholesterol) and were housed in colony cages in a temperature-controlled environment (22°C-25°C) with a 12 h light/dark cycle. In the first experiment, mice (N=17) were randomized to one of three dietary groups: control ((n=5) 0.1% choline wt/wt and 0% betaine wt/wt), choline-supplemented ((n=6) with 1% choline wt/wt, 0% betaine wt/wt), or betaine-supplemented ((n=6) 0.1% choline wt/wt, 0.9% betaine wt/wt). In the second feeding trial, mice (N=15) were randomized to one of two dietary groups: control ((n=7) 0% TMAO wt/wt) or TMAO supplemented ((n=8) 0.2% TMAO wt/wt). After the 8-week dietary intervention, the animals were euthanized, and tissues and blood collected. Aortic atherosclerotic plaque area, plasma choline and lipid metabolites were quantified. Liver histology and lipids were analyzed.

Results: In the first feeding trial, we observed that a choline-supplemented diet reduced atherosclerotic plaque area versus control diet, while a betaine-supplemented diet did not affect the lesion size. Interestingly, plasma TMAO levels were increased by choline supplementation as compared to the control diet; however, there was no change in plasma cholesterol, triglycerides, choline, betaine or TMA between dietary groups. Also we found, in the liver, a significant increase of phosphatidylcholine (PC) and an increasing trend in phosphatidylethanolamine (PE) in the choline-supplemented group as compared to control; however, there was no significant difference in PC/PE. Hepatic TG levels tended to be lower in the choline- and betaine-supplemented groups as compared to control animals. Weight gain, liver weight, and white adipose tissue weight were not altered between dietary groups. From the second feeding trial, we found, to our surprise that TMAO supplementation did not increase atherosclerosis even though there was a significant increase in plasma TG, TMA and TMAO levels. TMAO supplementation did not alter plasma cholesterol or hepatic TG, PC, and PE levels.

Conclusion: The results from our pilot were surprising: Ldlr−/− mice fed a choline-supplemented diet show a reduction in the atherosclerotic plaque area, despite having elevated plasma TMAO levels. Furthermore, TMAO supplementation increase plasma TMAO by 4-fold but did not influence atherosclerotic plaque area. Future work is required to understand the relationship between dietary choline and atherosclerosis in the Ldlr−/− mouse model.

Keywords: Choline, TMAO, Atherosclerosis, Ldlr
EXAMINING SEX DIFFERENCES IN GLYCEMIC INDEX KNOWLEDGE AND INTAKE AMONG INDIVIDUALS WITH TYPE 2 DIABETES

Hayford M. Avedzi1; Nonsikelelo Mathe1,2; Kate Storey1; Jeffrey A. Johnson1; Steven T. Johnson2

1 School of Public Health, University of Alberta, Edmonton, Alberta Canada
2 Centre for Nursing and Health Studies, Faculty of Health Disciplines, Athabasca University, Athabasca, Alberta Canada

Background: Dietary carbohydrates significantly influence glucose-insulin homeostasis, cardiometabolic risk factors, and health outcomes for people with type 2 diabetes. The Glycemic Index (GI) concept emphasises overall diet quality and has been recommended for guiding food selection among people living with diabetes. We examined self-reported dietary behaviours and actual food intakes among adult men and women with type 2 diabetes participating in Alberta's Caring for Diabetes (ABCD) Study.

Methods: For this cross-sectional study, participants completed a 3-day food record and questions about GI concept knowledge and low-GI dietary behaviours. Daily average GI and Glycemic Load were also calculated for all dietary carbohydrates consumed. Dietary intake was analyzed using ESHA FoodPro (version 10.13.1). Differences in nutrient intakes across categories of GI knowledge and low-GI dietary practices were explored by sex.

Results: Participants (N=170) mean (SD) age 65.8 (9.6) years were 46.5% women, 90.6% Caucasian with a mean BMI of 31.3 (7.0) kg/m² and diabetes duration of 13.4 (8.6) years. Overall, 60% and 40% of men and women respectively consumed carbohydrates in quantities below Acceptable Macronutrient Distribution Ranges (AMDR). Among men, 80% and 60% consumed proteins and fats respectively in quantities exceeding AMDR. Similarly, 90% and 65% of women consumed proteins and fats in quantities above AMDR. Fibre intake among men was lower than recommended (p<0.01). Men who reported having knowledge of the GI-concept also reported lower GI intake versus men who did not (p=0.03).

Conclusion: Sex differences exist in low-GI diabetes self-care dietary behaviours among adults with type 2 diabetes participating in this study. Gender-sensitive approaches for enhancing diabetes self-care low-GI dietary behaviour should be explored.

Key words: Glycemic Index, Dietary Intakes, Type-2 Diabetes
IMPROVING ISLET SURVIVAL BY ELEVATION OF INTRA-ISLET GLP-1

Campbell S, Ondrusova K, Barr A, Long W, Fatehi M, Light P

Department of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta

Background: Therapies that mimic or augment the action of Glucagon-like peptide-1 (GLP-1) are successfully used to treat patients with type 2 diabetes. It remains to be explored if these therapies will be useful in the treatment of type 1 diabetes, particularly in the preservation and maintenance of beta cell mass in islet transplantation. Islets secrete intra-islet GLP-1 from alpha cells in response to stress and express the enzyme that degrades GLP-1, Dipeptidyl peptidase-4 (DPP-4). There are a number of DPP-4 inhibitors, such as Sitagliptin, on the market for the treatment of type 2 diabetes that increase GLP-1 levels secreted from the gut in response to a meal. These inhibitors should also increase levels of active intra-islet GLP-1 and localize the anti-apoptotic and proliferative properties of GLP-1 to the islet beta cells. This study uses in vitro methods to explore a mechanism that may already be at work with current DPP-4 inhibition in type 2 diabetes and looks at the potential for its use in islet transplantation. I hypothesize that intra-islet GLP-1 can be elevated with DPP-4 inhibitors to increase islet survival.

Methods: Mouse and human islets are cultured at 6.1mM glucose with or without Sitagliptin 200nM. Active GLP-1 is measured in media samples at time 0, 8, 16, and 24h using a chemiluminescent immunoassay. The dead cell assay for mouse and human islets uses serum withdrawal and Interleukin–1beta (50-100ng/ml) to induce cell death. Cell death is detected with Sytox Green where images are taken of the islets with an EVOS microscope (10X objective). Images are quantified for cell death with ImageJ using a thresholding technique.

Results: Active GLP-1 levels are increased when mouse and human islets (normal and type 2 diabetic) are cultured with Sitagliptin 200nM for 24h. Active GLP-1 levels are also increased when human islets are cultured in serum-free media indicating the presence and activity of islet-derived DPP-4. In mouse islets, Interleukin-1beta 50ng/ml increased active GLP-1 levels in the presence of Sitagliptin (P<0.05, N=3), but did not protect from cell death. Preliminary data in human islets suggest that treatment with Sitagliptin 200nM may be protective (N=1).

Conclusion: Inhibition of islet DPP-4 with Sitagliptin 200nM can elevate intra-islet GLP-1 levels by augmenting the regenerative response to stress and localizing the pro-survival effects of GLP-1 to the islets. However, the elevation of intra-islet GLP-1 did not protect mouse islets from cell death in in vitro culture, but preliminary data with human islets does suggest a protective effect.

Key words: Dipeptidyl peptidase-4, Glucagon-like peptide-1, islet survival
CONTRIBUTION OF KV2.1 CLUSTERING TO INSULIN EXOCYTOSIS AND BETA-CELL DYSFUNCTION IN TYPE 2 DIABETES.

Jianyang Fu, Xiaoqing Dai, Greg Plummer, Kunimasa Suzuki, John Githaka, Jocelyn Manning Fox, Christopher Newgard, Nicolas Touret, Patrick MacDonald

Department of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta

Background: Exocytosis of insulin-containing secretory granules from β-cells of pancreatic Islet of Langerhans is tightly regulated by ion channels, which control excitability and calcium influx. Channels also play an increasingly appreciated role in membrane micro-domain structure. Kv2.1, which mediate the repolarization of beta-cell action potentials in human and rodents, may also play a non-electrical role in exocytosis by direct interaction with Syntaxin1A at the channel C-terminus. Recently, an unique character of this channel has been reported. Kv2.1 can target to distinct membrane domains or clusters in neuronal cells. It has been confirmed that clusters require a downstream region of the channel C-terminus that does not overlap with the Syntaxin-binding domain. Interestingly, we find that, similar to neuronal cells, Kv2.1 are also mainly localized in small groups (clusters) that corresponds to sites at which insulin granules attach to the plasma membrane in both human β-cells and insulinoma cells. Here we have examined the role for Kv2.1 clusters as facilitators of insulin exocytosis in pancreatic beta-cells from both non-diabetic human donors and donors with type 2 diabetes (T2D).

Methods: Patch-clamp measurement of currents and exocytosis were performed following knockdown of endogenous channels or up-regulation of recombinant channels. Super resolution and Total internal reflection fluorescence microscopy (TIRFM) examined clustering of Kv2.1 channels, and their co-localization with secretory granules, in combination with biochemical approaches to detect clustering and protein-protein interactions.

Results: Kv2.1, but not the related Kv2.2, functions as a direct facilitator of insulin exocytosis and forms clusters of ~8 channels at the plasma membrane. These co-localize with Syntaxin 1A clusters and membrane-resident secretory granules, and increase in density upon glucose stimulation. Kv2.1 mRNA is reduced by 80% in T2D islets, and membrane-associated Kv2.1 clusters are decreased in T2D beta-cells. Impaired exocytosis in T2D beta-cells can be rescued by up-regulating Kv2.1, which increases secretory granule recruitment. Finally, a clustering-deficient Kv2.1 mutant (Kv2.1-delta318) retains electrical function and syntaxin 1A binding, but does not enhance granule recruitment and exocytosis.

Conclusion: The ability of Kv2.1 to directly facilitate insulin exocytosis depends on channel clustering that is, in part, glucose-dependent. This suggests an important structural role for the channel at the exocytotic site, which may contribute to impaired insulin secretion when Kv2.1 expression is reduced in T2D.

Key words: Clusters, Exocytosis, Insulin granule, Kv2.1
ENDOPLASMIC RETICULUM (ER) STRESS CHAPERONE PROTEINS AND ASSOCIATED INSULIN COMPENSATION IN NILE RATS: AN EMERGING MODEL FOR TYPE 2 DIABETES

Hui Huang, Kaiyuan Yang, Yves Sauvé, Catherine Chan
Department of Physiology, Faculty of Medicine and Dentistry, University of Alberta

Background: Endoplasmic reticulum (ER) stress in pancreatic β-cells, induced by misfolded protein accumulation, is considered part of the pathogenesis of human type 2 diabetes (T2D); however, the role of ER stress proteins in β-cell function as changes in insulin secretory capacity occur is not clearly elucidated and some authors suggest that they also play a role in β-cell adaptation early in diabetes development. The Nile rat (NR) is an emerging model of spontaneous T2D, which progresses slowly and which appears to mimic the stages of human T2D. Studies in our lab revealed altered accumulation of (pro) insulin in NR β-cells due to increase of insulin demand in 12-month-old (12mon) NR, which may induce ER stress. Based on that, we hypothesize that ER stress proteins are elevated in NR, contributing to its diabetic characteristics.

Methods: NR were fed with high energy, low fibre (chow) diet (fat 9.6%, fiber 3.2% w/w) or low energy, high fibre (Hfib) diet (fat 4.1%, fiber 15.0% w/w) after weaning. Animals at age of 2mon, 6mon or 12mon were fasted overnight before tissue collection. Fasting blood glucose (FBG) and plasma insulin (FPI) were measured by glucometer and ELISA, respectively. Visualization of ER proteins was based on ER protein-insulin double-staining immunofluorescence (IF) protocol. Images were taken using a confocal microscope and colocalization of ER proteins with insulin quantified using Volocity.

Results: NR fed with chow diet developed hyperinsulinemia at 2mon (3.2 ± 0.9 ng/ml, p<0.05 compared with Hfib) and then hyperglycemia (17.6 ± 1.8 mM, p<0.001) but reduced insulinemia at the age of 12 months, whereas Hfib NR exhibited a mild upward trend in FPI but stable FBG (remaining <5 mM) with age. Three ER stress markers were measured by IF: protein disulfide isomerase (PDI), ERp44 and ERp72. PDI in NR increased at 2mon then decreased sharply after 6mon, but was maintained at a low level in Hfib group throughout. Positive correlation of insulin co-localized PDI with FPI was found in chow NR (r > 0.5, p <0.05). ERp44 IF intensity was significantly up-regulated (p< 0.05) in chow NR at 6mon and 12mon. ERp72 production presented an aged-related pattern in β cells, with relatively high intensity prior to 6mon but decreasing at 12mon in both chow and Hfib groups.

Conclusion: NR fed chow diet developed T2D with hyperglycemia and hypoinsulinemia, which was prevented by diet modulation. The ER chaperone protein exhibited distinct staining patterns as hyperglycemia developed and insulin secretion exhibited adaptation followed by decompensation, indicating the chaperones may not only be involved in T2D progression but may also play different roles in the adaptive and decompensation phases of insulin secretion in NR.

Key words: Nile rats, type 2 diabetes, ER stress chaperones
PROLIFERATION RESPONSE OF PBMCs FROM HUMAN DONORS WITH OR WITHOUT TYPE 1 DIABETES MELLITUS AGAINST NEONATAL PORCINE ISLET CELLS IN VITRO

Wenlong Huang, Ping Wu, Gina R. Rayat

Alberta Diabetes Institute, Ray Rajotte Surgical-Medical Research Institute, Department of Surgery, Faculty of Medicine and Dentistry, University of Alberta

Background: Islet transplantation is being considered as an alternative treatment for type 1 diabetes mellitus (T1DM). However, the shortage of human organ donors limits its wider application in the clinic. Researchers in the field are exploring the potential of neonatal porcine islets as an alternative source. The immune mediated rejection of porcine islets by human immune cells is a major hurdle for the successful application of porcine islet xenotransplantation in patients with T1DM. In this study, we investigated the human immune cell mediated response against neonatal porcine islet cells in vitro.

Methods: Peripheral blood mononuclear cells (PBMCs) from human donors with T1DM or without T1DM and from 3-day-old wild type neonatal pigs were isolated following our standard protocol. Wild type neonatal porcine islets were isolated and cultured for 7 days, and then dissociated into single islet cells. One-way mix lymphocyte reactions were performed in 96-well plates, in which human PBMCs were used as responder cells (5×10^5 cells per well) while gamma irradiated porcine islet cells and porcine PBMCs served as stimulator cells (3×10^5 cells per well). Concanavalin A (ConA) was also used to stimulate the proliferation of human PBMCs and response from these cells served as a positive control. On day 1 to day 7 of culture, 10 µl of [3H]-thymidine was added to each condition, the cells were further incubated for 24 hours and proliferation of responder cells was detected using a gamma counter and expressed as counts per minute (CPM).

Results: Without stimulation, human PBMCs from donors with or without T1DM had limited proliferation (1598±409 CPM vs 1160±390 CPM). When treated with ConA, maximum proliferation of human PBMCs from individuals with and without T1DM was observed on day 3. However, the proliferation of PBMCs from individuals without T1DM was significantly (p=0.020) higher (39,383±5,545 CPM, n=4) compared to the proliferation of PBMCs from individuals with T1DM (22,846±7,016 CPM, n=6). When PBMCs from individuals without T1DM were stimulated with porcine PBMCs, maximum proliferation was observed on day 7 (40,130±4,850 CPM), while PBMCs from individuals with T1DM reached maximum proliferation on day 7 (19,200±1,941 CPM), and was significantly (p<0.001) lower compared to the response observed in individuals without T1DM. When PBMCs from individuals with T1DM were stimulated with porcine islet cells, maximum proliferation was observed on day 7 (9,838±1,894 CPM), which was significantly higher (p=0.034) compared to the proliferation of PBMCs from individuals without T1DM (4,632±1,583 CPM), which reached maximum response on day 6.

Conclusion: PBMCs from individuals with T1DM showed more robust proliferation when stimulated with ConA and porcine islet cells, but not porcine PBMCs compared to the proliferation of PBMCs from individuals without T1DM. This may reflect the beta cell specific auto-immune condition of the human donors.

Key words: Mixed Lymphocyte Reaction, Type 1 Diabetes Mellitus, Non-diabetic Individual
RESCUE MECHANISMS FOR KATP LOSS OF FUNCTION MUTATIONS HIGHLIGHT ESSENTIAL RESIDUES AT THE KIR6.2 CHANNEL DOMAIN INTERFACE

Jenny B. Li, Robin Y. Kim, Runying Yang, Harley T. Kurata
Dept. of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta

Background: ATP-sensitive potassium (KATP) channels are essential transducers of cellular metabolism that initiate the electrical signals leading to insulin secretion. Genetic defects of KATP channel genes translate into altered protein function leading to insulin release disorders (diabetes or hyperinsulinism). We have investigated the molecular basis of KATP channel function and potential mechanisms underlying channel defects that arise from certain disease-causing mutations.

Methods: We used a combination of molecular modeling, inside-out patch clamp electrophysiology, and biochemistry to assess and interpret the effects of channel mutations on trafficking and gating function.

Results: Inspection of the architecture of Kir channels reveals a non-covalent interface between the 'ligand-sensing' C-terminal domain (CTD) and the canonical pore-forming 'gating domain' formed by the TM1 and TM2 transmembrane helices. This interface is involved in transduction of ligand binding, and intact coupling between the CTD and TMD of Kir6.2 channels has been proposed to be crucial for propagation of gating effects induced by intracellular ligands. However, many mutations in this interfacial region prevent channel function and thereby preclude functional studies to interrogate the domain interface. We have developed a novel rescue mechanism that circumvents this problem, and we have applied this method to scan the functional contributions of residues in the CTD-TMD interface of Kir6.2 channels.

Conclusions: Residues cluster into three distinct functional types, based on their impact on ATP sensitivity and gating kinetics of the F168E background mutation used for functional rescue. Most loss-of-function mutations are classified as 'efficiently coupled', and their ATP sensitivity is only modestly affected. A second group of mutations comprises residues in the ATP binding site, which are 'efficiently coupled' but are profoundly insensitive to ATP. Most interestingly, our studies reveal a subset of 'uncoupling' mutations that form a network between the C-linker, G-loop, C-D loop, and slide helix of the channel. These distinguish unique gating roles for residues that are required for channel function, and highlights the importance of a rescue approach to understand the impact of loss of function mutations.
BLUE LIGHT (470 nm) INDUCES PHENOTYPIC AND FUNCTIONAL CHANGES IN ADIPOCYTES VIA A MELANOPSIN-TRPC CHANNEL SIGNALING PATHWAY

Katarina Ondrusova, Mohammad Fatehi, Amy Barr, Zosia Czarnecka, Wentong Long, Scott Campbell, Peter Light
Light Laboratory, Department of Pharmacology, Alberta Diabetes Institute

**Background:** Exposure to ambient light regulates various biological processes such as circadian rhythms. This phenomenon is mediated by blue light activating the photoreceptor melanopsin that is coupled with TRPC channels in intrinsically photosensitive retinal ganglion cells. The stimuli regulating adipocyte physiology and functions are not well understood. Due to its proximity to light exposure, however, subcutaneous white adipose tissue may be directly affected by light stimuli by expression of melanopsin. As indication of this, we have discovered the presence of an endogenous blue light (470 nm)-sensitive current in 3T3-L1 mature adipocytes, that is eliminated in the absence of light stimulation.

**Methods:** Expression of OPN4, the gene encoding melanopsin, as well as TRPC channel variants 1,3,5 was determined using PCR in 3T3-L1 mature adipocytes and human subcutaneous white adipose tissue. Whole cell patch clamp was used to detect current in 3T3-L1 mature adipocytes and human subcutaneous mature white adipocytes during stimulation with pulsed blue (470 nm) light. In these experiments, opsinamide (melanopsin antagonist), U73122 (phospholipase C inhibitor), and clemizole (TRPC channel inhibitor) were used as pharmacological tools to delineate the mechanism of action. Glycerol release and Oil Red O staining were used to assess changes in lipid homeostasis in response to chronic blue light exposure over 13 consecutive days in 3T3-L1 mature adipocytes.

**Results:** Melanopsin is expressed in 3T3-L1 mature adipocytes and in purified human subcutaneous white adipocytes. TRPC1,3,5 are present in 3T3-L1 mature adipocytes and TRPC 1 and 3 are expressed in human subcutaneous white adipose tissue. Whole cell patch clamp data reveals a 470 nm light-induced current in 3T3-L1 mature adipocytes, that is significantly reduced in the presence of opsinamide, U73122, and clemizole. In human adipocytes, the light-induced current is also inhibited by opsinamide. Intriguingly, chronic blue light exposure causes a reduction in the number of cells retaining lipid droplets as well as an increase in glycerol release, indicating increased lipolysis.

**Conclusion:** Thus far, experiments suggest stimulation of melanopsin at the adipocyte cell membrane, followed by transduction of the signal by involvement of a TRPC channel variant in response to blue light. Furthermore, blue light seems to play a role in mediating lipolysis within the adipocyte. These findings suggest then that ambient light exposure may regulate various aspects of adipocyte function such as lipid homeostasis, and possibly adipokine secretion, or clock gene regulation. As adipocyte physiology remains in many ways undetermined, this is an exciting, novel area of study that may further propagate our understanding of this highly complex tissue and its function in pathophysiology.

**Keywords:** light, adipocyte, melanopsin, lipolysis
CREATINE SUPPLEMENTATION IN COMBINATION WITH RESISTANCE TRAINING IMPROVES GLYCEMIC CONTROL IN THE ELDERLY

Camila Lemos Pinto¹,²*, Barbara de Moura Mello Antunes³, Fábio Santos Lira³, Aline Corado Gomes², Gustavo Duarte Pimentel², João Felipe Mota².

Currently supervised by Dr. Carla Prado¹

¹Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada. ²Clinical Nutrition and Sports Nutrition Research Laboratory, Faculty of Nutrition, Goiania Federal University, Goiania, GO, Brazil. ³Exercise and Immunometabolism Research Group, Department of Physical Education, Sao Paulo State University, Presidente Prudente, SP, Brazil.

Background: Impairment of glucose and insulin metabolism with age can lead to type 2 diabetes mellitus. Aging is also associated with loss of lean mass, which has been linked to several factors including inflammation and insulin resistance. Evidence suggests resistance training alone, or in combination with creatine supplementation may have a positive therapeutic impact on lean mass and metabolic disturbances in glucose metabolism. Thus, the aim of this study was to investigate whether creatine supplementation in combination with resistance training was able to improve glycemic control in elderly individuals.

Methods: In a 12-week, parallel-group, double-blind, randomized, placebo-controlled trial, n=27 individuals (67.2 ± 5.5 years) were allocated into one of the following groups: placebo plus resistance training (PL + RT) or creatine supplementation plus resistance training (CR + RT). Participants from CR + RT group received 5 g/day of creatine monohydrate versus the same dose of maltodextrin for the PL + RT group, while engaged in a 12-week supervised resistance training program three times per week. At baseline and week 12, lean mass was assessed via DXA and blood samples were collected for analysis of glucose, insulin, adiponectin, brain-derived neurotrophic factor, interleukin 6, interleukin 10 and monocyte chemoattractant protein-1.

Results: Fourteen participants from PL + RT group and 13 from CR + RT group completed the trial. Compared to the PL+RT group, blood glucose levels were lower in the CR + RT group after the 12-week (104.4 ± 29.3 mg/dL and 91.5 ± 18.9 mg/dL, respectively; p<0.05) and lean mass gain was higher (0.6 ± 1.3 kg and 1.8 ± 1.3 kg, respectively; p=0.02). No other differences were observed between groups. An inverse correlation between lean mass with insulin (-0.64, p=0.01) and HOMA-IR (-0.69, p<0.01) was observed in the CR + RT group.

Conclusion: Twelve weeks of low-dose creatine supplementation in combination with resistance training resulted in decreased blood glucose levels in the elderly individuals. Moreover, lean mass gain was associated with lower insulin resistance in the supplemented group.

Key words: Creatine, elderly, exercise, glucose, insulin resistance, inflammation.
RANOLAZINE TREATMENT IMPROVES GLYCEMIA AND DECREASES BODY WEIGHT IN OBESE AND INSULIN RESISTANT MICE

Rami Al Batran, Keshav Gopal, Hanin Abursayn, Amina Eshreif, Wayne Ma, Farah Eaton, and John R. Ussher

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta

Background: Obesity-associated insulin resistance and type 2 diabetes (T2D) is a global health problem with increasing prevalence, which results in significant cardiovascular morbidity and mortality. As a result, new guidelines have been placed for manufacturers developing new therapies for T2D, as they must demonstrate that their therapy is cardiovascular safe. Ranolazine is an approved anti-anginal agent that is associated with reductions in glycemia in both patients with angina and/or T2D. Thus, ranolazine might be uniquely situated as a therapy that can reduce both hyperglycemia and cardiac dysfunction in T2D, and our aim was to confirm this in a mouse model of obesity and insulin resistance.

Methods: 10-week old C57BL/6J male mice were fed either a low-fat or high-fat diet for 10 weeks and then treated for 4 weeks with ranolazine (50 mg/kg via daily subcutaneous injection). Glycemic control was monitored in mice via glucose and insulin tolerance testing, whereas in vivo metabolism was assessed via in direct calorimetry.

Results: Chronic high-fat feeding induces obesity, insulin resistance, and altered substrate preference in male C57BL/6J mice. Interestingly, treatment with Ranolazine reduced body weight and improved glucose homeostasis in obese mice as indicated by enhanced glucose clearance during glucose tolerance testing. Furthermore, Western blot analysis demonstrated that ranolazine improves insulin-stimulated phosphorylation of glycogen synthase kinase-3 β in the soleus muscle and hearts of obese mice. The improved glycemia in obese mice following ranolazine treatment does not appear to be dependent on body weight reduction, as lean mice treated with ranolazine also showed enhanced glucose clearance following a glucose tolerance test. Last, the ranolazine-mediated reduction in body weight was independent of changes in food intake, but was associated with increased energy expenditure.

Conclusion: Our data demonstrate that treatment with ranolazine improves glucose homeostasis and reduces body weight in obese and insulin resistant mice, suggesting that ranolazine might be an ideal therapy for T2D patients at risk of cardiovascular disease.

Key words: Ranolazine; Obesity; Insulin Resistance; Glucose Homeostasis; Type 2 Diabetes
THE TYROSINE PROTEIN KINASE LYN: A NOVEL REGULATOR OF BETA-CELL PROLIFERATION AND SURVIVAL

Nidheesh Dadheec, Ying Wayne Wang, Bahareh Nemati Moud, Nik Wasylyk and Jean Buteau

Department of Agriculture Food and Nutritional Sciences, Faculty of Agricultural Life and Environmental Sciences, University of Alberta.

Background: Lyn is a member of the Src family of protein tyrosine kinases. Our previous study, investigating GLP-1 signaling in beta-cells, revealed a key role for a Src member in the regulation of beta-cell mass. We herein sought to precisely identify the Src family member that was mediating GLP-1 action, and examine its role in the regulation of beta-cell “life and death”.

Methods: We measured the expression and activity of Src family members in INS cells and human islets. We also performed gain- and loss-of-function experiments for each Src member, using virus-mediated overexpression and siRNA-mediated knockdown. We further confirmed the hitherto uncharacterized role of Lyn in the control of beta cell proliferation and apoptosis using a pharmacological approach.

Result: Our results show that pancreatic beta-cells express three Src family members: c-Src, Lyn and Fyn. Of those, only c-Src and Lyn were activated by the beta-cell growth factor GLP-1. Overexpression and down-regulation of c-Src, Lyn and Fyn unraveled a unique role for Lyn in beta-cell proliferation and survival. Indeed, knockdown of Lyn provoked beta-cell death, whereas Lyn overexpression prevented apoptosis in both INS cells and human islets. Similarly, knockdown of Lyn blunted INS cell proliferation, whereas Lyn overexpression resulted in increased proliferation. Pharmacological activation of Lyn using MLR1023 reiterated the effects of Lyn gain-of-function, in addition to increasing insulin release.

Conclusion: Our study identifies and characterizes Lyn as a novel regulator of beta-cell mass and function. As such, Lyn could represent a promising molecular target for diabetes treatment.

Keywords: Lyn, c-Src, tyrosine protein kinases, beta-cells, proliferation, apoptosis.
OBJECTIVELY MEASURED DAILY PHYSICAL ACTIVITY AND SEDENTARY TIME IN WOMEN WITH PREVIOUS GESTATIONAL DIABETES MELLITUS

Nonsikelelo Mathe1,2, Abdulrhman Alghamdi3, Margie Davenport4, Sonia Butalia5,6 Jeffrey A. Johnson1, Steven T. Johnson1,2
1 Alliance for Canadian Health Outcomes Research in Diabetes, School of Public Health University of Alberta, 2 Faculty of Health Disciplines, Athabasca University, 3 Faculty of Science, University of Alberta, 4 Faculty of Physical Activity and Recreation, University of Alberta, 5 Division of Endocrinology, University of Calgary, 6 Alberta Health Services

Background: Gestational diabetes mellitus (GDM) increases the risk of developing type 2 diabetes after pregnancy. Increased physical activity may aid in reducing the risk type 2 diabetes after GDM. However, women with a previous GDM pregnancy are unlikely to participate in regular and sufficient physical activity for health. In this study, physical activity was measured objectively using accelerometry.

Methods: Women previously diagnosed with GDM (n=30) participating in the Healthy Eating and Active Living for Gestational Diabetes (HEALD-GDM) answered questions on social demographics, lifestyle and physical activity and wore an accelerometer (Actigraph®GT3X+) around their waist for seven consecutive days during waking hours. Physical activity (i.e., light intensity (LPA) and moderate to vigorous (MVPA)) and sedentary time were described, and linear regression was used to investigate correlates of these behaviours with socio-demographic characteristics.

Results: Participants were on average 35.4 years of age [standard deviation (SD) 4.4], mean body mass index was 30.5 (10.2) kg/m2, and HbA1c 5.5 (0.3)% waist circumference 95.1 (15.5) cm. The majority were married (100%) had college education or higher (73%), were of Caucasian ethnicity (87%), had a household income >$100000 annually (63%), and were employed full-time (63%). Accelerometry data were available for participants who wore the accelerometer for an average of 6.4 (4.6) days. On average, participants were sedentary for 273.0 minutes/day or 54.1% of the day. They spent 217.7 minutes/day in LPA and 38.1 minutes/day in MVPA. Women who were employed had more sedentary time 67 minutes/day (95% CI(18.09, 115.03) and significantly less LPA time -67 minutes/day 95% CI -109.93; -23.24). Women whose household income was less than $40,000/ annum had more MVPA time.

Conclusion: Women who had GDM in pregnancy were highly sedentary. Those employed and on higher incomes among this population may require specific targeting to increase MVPA and reduce overall sedentary time.

Key words: Physical activity, Gestational diabetes mellitus, Type 2 diabetes, accelerometer
RESTORING HEAT SHOCK FACTOR 1 ACTIVITY TO PREVENT BETA-CELL APOPTOSIS

Indri Purwana (1), Junjun Liu (2), Bernard Portha (2), Jean Buteau (1)

(1) Department of Agriculture, Food, and Nutritional Science, University of Alberta
(2) Unit of Functional and Adaptive Biology, Paris-Diderot University

Background: Heat Shock Factor 1 (HSF1) is a transcription factor that regulates the expression of key molecular chaperones heat shock proteins (HSPs), thereby orchestrating cellular response to stress. This system has recently been implicated in the control of insulin sensitivity and is therefore scrutinized as a novel therapeutic avenue for type 2 diabetes. However, the biological actions of HSF1 in beta-cells remain elusive. We herein sought to investigate the regulation of HSF1 in pancreatic beta-cells and to study its potential role in survival.

Methods: We measured the expression of canonical targets of HSF1 in vitro and in islets from diabetic Goto-Kakizaki (GK) rats. To study HSF1 under metabolic stress in beta-cells, we exposed human islets and INS1 beta-cell line to glucolipotoxicity and thapsigargin. HSF1 activity was evaluated by gel shift assay. HSF1 acetylation and interaction with the protein acetylase CREB-binding protein (CBP) were measured by western blot. We delineated the effects of HSF1 acetylation using mutants mimicking constitutive acetylation and deacetylation of the protein. Cell death was measured by TUNEL assay following HSF1 overexpression.

Results: The expression of HSF1 and its target genes were decreased in islets from diabetic GK rats, suggesting the possible role of HSF1 in the pathophysiology of diabetes. Glucolipotoxicity induced HSF1 acetylation and interaction with CBP. Glucolipotoxicity-induced HSF1 acetylation inhibited its DNA binding activity and decreased the expression of its target genes. Restoration of HSF1 activity in beta-cells prevented glucolipotoxicity-induced ER stress and apoptosis. However, overexpression of a mutant protein (K80Q) mimicking constitutive acetylation of HSF1 failed to confer protection against glucolipotoxicity.

Conclusion: Our results unravel a new mechanism by which glucolipotoxicity inhibits HSF1 activity to cause beta-cell apoptosis. Restoring HSF1 activity may represent a novel strategy for the maintenance of a functional beta-cell mass. Our study supports the therapeutic potential of HSF1/HSPs targeting agents in diabetes treatment.

Keywords: heat shock protein, acetylation, ER stress, glucolipotoxicity
RAPID OPTIMIZATION AND SCALE-UP OF CELL-BASED THERAPIES FOR TYPE 1 DIABETES

Derek Toms, Doug Kondro, Yang Yu, Mark Ungrin
Department of Comparative Biology and Experimental Medicine, Faculty of Veterinary Medicine, University of Calgary

BACKGROUND: While exogenous insulin delivery can palliate the effects of type 1 diabetes, cell-based therapies seeking to replace lost beta cells show promise in animal models and clinical trials. These therapies primarily employ the directed differentiation of human pluripotent stem cells (PSCs), precursor cells capable of self-renewal, to generate early stage beta cells for transplantation. This differentiation process involves complex, multistep protocols that have been developed based on developmental paradigms and iterative refinements. As a result, these exciting advances are hampered by limited efficiency of the techniques, and difficulty in adoption by other researchers. Furthermore, the prediction that a 70-kg patient with diabetes will require up to a billion insulin-producing cells to effectively normalize blood glucose highlights the need for the ability to scale-up production once efficient cell-based therapies are devised.

METHODS: Building on our AggreWell system, a novel microwell technology for the ultra-high throughput generation of engineered cellular aggregates, we have begun developing a platform for optimizing differentiation protocols and a microwell bioreactor to allow seamless scale-up to produce quantities of cells necessary for in vivo research trials or therapeutic applications. We incorporate statistical Design of Experiments (DoE) methodology, a formalized process that maximizes the amount of information that can be gained from a limited number of experimental data points.

RESULTS: We have successfully produced 96-well microplate microwell surfaces to use for high-throughput applications. Paired with this, our prototype microwell bioreactors are capable of generating 50 000 aggregates in a single experimental run (compared to 200 per well of the 96-well microplate). Validation of this platform directing the differentiation of PSCs to definitive endoderm, an initial pancreatic precursor cell type, showed a nearly 15% increase in yield in an optimized protocol. Importantly, we also show that differentiation using the AggreWell technology is scale-independent across three orders of magnitude. Finally, we have successfully included time variables in our optimization process to further increase PSC differentiation efficiency.

CONCLUSION: By removing the need for empirical optimization when scaling up, protocols developed using microwell technology can be scaled to any production level requirements. By optimizing temporal cellular signaling to direct PSC differentiation, we plan to extend this work to the currently complex protocols necessary for generating mature pancreatic beta cells. By developing the associated bioreactor technology in parallel, we provide a platform upon which cell-based therapies for type 1 diabetes can be improved in an efficient and logical manner, with the ability for direct translation to in vivo testing.

KEYWORDS: pluripotent stem cells; directed differentiation; bioprocess optimization.
**TRANS-11 VACCENIC ACID IMPROVES INSULIN SECRETION VIA GPR40 IN RAT AND HUMAN MODELS OF TYPE 2 DIABETES**

Xiaofeng Wang, Gina Rayat, Spencer D. Proctor and Catherine B. Chan  
Department of Agricultural, Food and Nutritional Science, University of Alberta

**Background:** Trans-11 vaccenic acid (VA), a trans-fatty acid produced by ruminant animals, is not associated with cardiovascular risk in contrast to industrially-produced trans-fatty acids. Our previous study suggests that VA improves insulin secretion in type-2 diabetes (T2D) models *in vivo* and *in vitro*. VA also increased G-protein coupled receptor (GPR)40 expression, as measured by qPCR in both human and rat islets. GPR40 is a receptor for medium and long-chain free fatty acids and its activation promotes glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells and inhibits β-cell apoptosis. Thus, we hypothesized that VA improves insulin secretion via a GPR40-dependent mechanism.

**Methods:** VA was provided orally to Sprague Dawley rats at 10.5 g/Kg in the diet for a total of 12 weeks in the treatment group. T2D was induced in animals by high-fat diet combined with single low-dose streptozotocin (STZ) injection in the 5th week. Gene and protein expression in isolated rat islets were determined by qPCR and western blot respectively. *In vitro*, isolated islets from normal rats and human islets were treated with 15 mM STZ + 0.5 mM palmitic acid (PA, for rat islets) or 10 ng/mL tacrolimus + 0.4 mM PA (for human islets) with or without 0.4 mM VA and 1 μM GW1100 (GPR40 antagonist). GSIS was measured 24 or 48 hours after treatments.

**Results:** GPR40 mRNA and protein abundance in islets were both higher in T2D+VA than T2D animals by 40-fold and 1.9-fold, respectively. *In vitro*, VA treatment significantly enhanced GSIS in response to 22 mM glucose in rat islets after STZ+PA challenge, an effect blocked by GPR40 antagonist, GW1100. Neither VA nor GW1100 had any effect on basal insulin secretion or islet insulin content. Similar results were observed in human islets challenged with tacrolimus + PA.

**Conclusion:** These results indicate that GPR40 plays a role in the promoting effect of VA on insulin secretion in the context of T2D.

**Key words:** Trans-11 vaccenic acid, Type-2 diabetes, Insulin secretion, GPR40
GLUCOSYLCERAMIDE SYNTHASE, A CRITICAL REGULATOR OF BETA-CELL SURVIVAL

Dassine Berdous, Cyndi Henry, Si Mi, Jonathan Curtis, Ying Wang, and Jean Buteau

Department of AFNS, Faculty of ALES, University of Alberta

Type 2 diabetes is characterized by a progressive deterioration of beta-cell mass and function. The causes of this loss in beta-cell mass are multifactorial and include chronic exposure to elevated concentrations of both glucose (glucotoxicity) and saturated fatty acids (lipotoxicity). However, the molecular mechanisms precipitating beta-cell demise remain elusive.

We previously demonstrated that the transcriptional repressor ST18 acted as a novel and prominent mediator of lipotoxicity. Importantly, the biological roles and transcriptional targets of ST18 have never been investigated in beta-cells.

We herein identify glucosylceramide synthase (Ugcg) as a target of ST18 in beta-cells. We show that ST18 binds a conserved responsive element in the Ugcg promoter to down-regulate Ugcg expression. ST18-mediated inhibition of Ugcg promotes the accumulation of ceramides and stunts their conversion into glucosylceramides, a detoxification pathway. Consistently, we also show that genetic and pharmacological inhibition of Ugcg provoke beta-cell apoptosis. Conversely, we demonstrate that overexpression of Ugcg is protective against apoptosis.

Altogether, these results characterize Ugcg as a critical regulator of beta-cell survival that is inhibited during lipotoxicity. Ugcg represents an interesting potential pharmacological target in diabetes treatment.

Keywords: Ugcg, ST18, lipotoxicity, apoptosis, beta-cell survival
2016 ADI RESEARCH DAY
Tuesday October 4
LISTER HALL, Maple Leaf Room, University of Alberta

NOTES
2016 ADI RESEARCH DAY
Tuesday October 4
LISTER HALL, Maple Leaf Room, University of Alberta

NOTES
2016 ADI Research Day
Tuesday October 4

Made possible through support by

MERCK

Lilly
Answers That Matter.

Alberta Diabetes Foundation