Thirteenth Annual Scientific Meeting
Montreal Diabetes Research Center

Theme: Genes and Environment In Cardiometabolic Health

February 1, 2019

Final Program

The retreat is supported by generous contributions from:

For clinician specialists this activity is recognized as continued education (one credit per keynote lecture for a total of two) by the Royal College of Physicians and Surgeons of Canada
Program

8:00 AM  Registration and light breakfast

8:30  Marc Prentki, MDRC Director
Introduction

Oral Presentations, Session 1

8:45  6th George F Cahill Jr Lecture
Elizabeth J Mayer-Davis, PhD, U of North Carolina, Chapel Hill
Nutrition and vascular disease prevention in Type 1 Diabetes:
Where are We Now?

9:45  Best Paper of the Year
Ariel M Wilson
Neuropilin-1 expression in adipose tissue macrophages protects
against obesity and metabolic syndrome.

10:00  Pause and photo

10:30  Pitch talks
MSc Students: Myriam Hoyeck, Abel Oppong
PhD Students: Valérie Lamantia, Mélissa Léveillé, Hasna Maachi,
Romane Manceau, Céline Schott
Post-Doc: Cristina Bosoi, Kana Miyata, Pegah Poursharifi

11:15  Kaberi Dasgupta, MD, MUHC, McGill
Supporting self-management in diabetes prevention and care.
11:45  **1st Martin Rodbell Translational Research Lecture**  
Jean-Claude Tardif, MD, ICM, UdeM  
*Curbing atherosclerosis with precision medicine.*

12:45  Buffet and Poster Session  
*Buffet will be held at CRCHUM Agora (5th floor)*

1:30– 3:30  Poster Session: Evaluation by the jury

**Oral Presentations, Session 2**

3:45  **André Carpentier, MD, U Sherbrooke**  
*Brown fat in cardiometabolic health.*

4:15  **14th J Denis McGarry Lecture**  
Nancy J Cox, PhD, Vanderbilt U, Nashville TN  
*Iterating between a curated sample of 700,000 with BMI trajectories over 5-year intervals and integrated -omics in a biobank to better understand genetic relationships among obesity, Type 2 Diabetes and its complications.*

5:15  **MDRC/Diabète Québec Pilot Grants**  
**MDRC Award for Best Oral & Poster Presentations**

5:30  **Cocktail and Posters**
Denis McGarry was born in Widness, England in 1940. He did his undergraduate and graduate work at the University of Manchester, receiving the Ph.D. in 1966. He did two years of postdoctoral fellowship at the University of Liverpool and the University College of Wales before joining Dan Foster’s lab at Southwestern Medical Center in Dallas as a postdoctoral fellow in 1968. He was appointed Assistant Professor of Internal Medicine in 1969 and reached full professorship in Internal Medicine and Biochemistry in 1997, at which time he was also named the Clifton and Betsy Robinson Chair in Biomedical Research.

Denis was a gifted teacher who was regularly judged outstanding by medical students that attended his lectures on metabolism in the Biochemistry course. He also taught in the graduate school and lectured Internal Medicine residents and Endocrine fellows.

Research, however, was his passion. He had an uncanny knack to make discoveries that changed the way that other scientists thought about metabolism. He defined the malonyl CoA regulatory system operating through carnitine palmitoyltransferase 1 (CPT1) and showed that the ketosis of starvation and the ketoacidosis of insulin-dependent diabetes was the consequence of a glucagon-induced fall in malonyl CoA. Solution to the problem of ketogenesis had eluded such illustrious names as Krebs, Wieland, and Lehninger. He subsequently showed that the malonyl CoA/CPT1 system operated in many other tissues. Under his leadership the laboratory cloned and sequenced the involved genes and unequivocally proved that CPT1 of liver was distinct from CPT1 of muscle and that CPT1 and CPT2 were separate enzymes derived from different genes.

He also devoted considerable energy to the mechanism by which glycogen was synthesized from glucose after a fast. In contrast to conventional wisdom, he showed that the indirect pathway, the Cori cycle (glucose → lactate → glucose-6-PO4 → glycogen) was dominant over the direct pathway (glucose → glucose-6 PO4 → glycogen).

In 1992, he published a famous review paper in Science (Science 1992; 258:766-770) entitled "What if Minkowski Had Been Ageusic? An alternative angle of diabetes". He suggested that scientific focus on abnormal glucose metabolism had masked the critical importance of abnormal fat metabolism, especially in type 2 diabetes mellitus.
Subsequent to this paper there was a huge swing by investigators toward the key role of abnormal lipid metabolism in insulin resistance and lipotoxic damage to tissues as diverse as the heart and the beta cell of the pancreas.

In late April 2001, Denis was diagnosed with glioblastoma multiforme after the sudden appearance of expressive aphasia. He received the 2001 Banting Medal for scientific achievement from the American Diabetes Association, but his health sadly prevented him from giving the lecture. It was given beautifully by ADA President Bob Sherwin who emphasized studies on the role of dysregulated fatty acid metabolism in the diabetic state. Denis felt blessed that he was able to be present and receive the medal.

In addition to the Banting Medal, Denis had previously received the Lilly Award, the Herman O. Mosenthal Award, the Joslin Medal, the David Rumbough Scientific Award and the Grodsky Award.

As his death approached, his friends wanted to raise money for a distinguished chair before he died. The size of some of the gifts from the faculty was astounding -$100,000. Pledges for a million dollar were quickly raised. The U of Texas Medical School normally gives an actual chair to the major donors, but the donors wanted Denis to have it. There was a reception in his home to award it and those present will never – ever – forget a classic scene. Denis was sitting in the chair and kneeling on the floor before him were Steve McKnight, Mike Brown, Joe Goldstein and another scientist who held his hand. It was incredibly touching. Denis McGarry died peacefully at his home in the presence of his family on the evening of January 27, 2002.

A remarkable thing about Denis was the vast number of deep friendships he had in the world of diabetes and the scientific community. He was extremely rigorous, pertinent and original in the way he approached a scientific problem, often starting from simple physiological observations leading to testable hypotheses. He acted like a magnet for young investigators who always wished to discuss informally with him and who much appreciated his original turn of mind with a vision of biology and physiology at large. His papers were extremely well written with a touch of literacy and perhaps the writer James Joyce that he admired so much, also native from Ireland, inspired him. Denis was a joyful person and he could test and tease you in a bar anywhere in the world by asking with a playful smile “what was the most important discovery and experiment in the field of diabetes?” Like everyone but with some doubts because of the triviality of the question you would answer that it is the discovery of insulin. He would smile again and say “Absolutely not! It is the removal of a dog pancreas by Oskar and the serendipitous observation of the flies attracted by the sweet urine…and not repelled by the fat-derived acetone”. Without saying he would win this lost battle for you with a little cigarette remain in his mouth, the smoke volutes going to heaven and an excellent glass of red Bordeaux in his hand….and as a compensation of you feeling so dumb, magnanimous he would offer you another glass. Denis was also humble and he would say “well besides Mozart, Einstein and few others among centuries who will remember what we did in a hundred years? ”
No comments about Denis McGarry would be complete without mentioning that he was a devout Roman Catholic.

At the beginning of the 7th century, Isadore, Archbishop of Seville, gave his prescription for a good life.

Learn as if you were to live forever.
Live as if you would die tomorrow.

Denis did both. He learned all his life in science. When death came, he was ready.

*Text written by Dan Foster and Marc Prentki*

**In the memory** of John Denis McGarry, the Montreal Diabetes Research Center is proud to organize each year “The J Denis McGarry lecture” given by world-leaders and outstanding speakers.

The J Denis McGarry Lecture 2017 will be given on February 3 at the CRCHUM by Dr. Scott M Sternson from the Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA. His lecture is entitled: *The Systems and Molecular Neuroscience of Hunger.*

Previous J Denis McGarry lecturers were:

<table>
<thead>
<tr>
<th>Year</th>
<th>Recipient</th>
<th>Title of the lecture</th>
</tr>
</thead>
<tbody>
<tr>
<td>2018</td>
<td>Susan Bonner-Weir</td>
<td>Pancreatic beta cell-mass maintenance and regeneration health, diabetes and aging.</td>
</tr>
<tr>
<td>2017</td>
<td>Scott M. Sternson</td>
<td>The Systems and Molecular Neuroscience of Hunger</td>
</tr>
<tr>
<td>2016</td>
<td>C. Ronald Kahn</td>
<td>Interactions between genes, environment and the gut microbiome in insulin resistance and metabolic syndrome.</td>
</tr>
<tr>
<td>2015</td>
<td>Philipp Scherer</td>
<td>Diabetes, Obesity and the Central Role of the Adipocyte Maintaining Systemic Homeostasis.</td>
</tr>
<tr>
<td>2014</td>
<td>Stephen Woods</td>
<td>Metabolic Peptides, Food Intake and Body Weight: Problems with the Model.</td>
</tr>
<tr>
<td>2013</td>
<td>Steven Kahn</td>
<td>The Beta Cell in Type 2 Diabetes: Is She Still the Main Culprit?</td>
</tr>
<tr>
<td>2012</td>
<td>Steven McKnight</td>
<td>Unique Dependence of Mouse Embryonic Stem Cells on Threonine Catabolism</td>
</tr>
<tr>
<td>2011</td>
<td>Juleen Zierath</td>
<td>Gene/Environmental influence on skeletal muscle insulin sensitivity</td>
</tr>
<tr>
<td>2010</td>
<td>Bruce Spiegelman</td>
<td>Regulation of Brown Adipogenesis: Mechanisms Therapeutics</td>
</tr>
<tr>
<td>2009</td>
<td>Domenico Accili</td>
<td>Understanding β-cell failure: lessons from Foxo biology</td>
</tr>
<tr>
<td>2008</td>
<td>Gokhan S Hotamisligil</td>
<td>Inflammatory basis of metabolic diseases</td>
</tr>
<tr>
<td>2007</td>
<td>Rudolph L Leibel</td>
<td>Quantifying pancreatic β-cell mass in vivo in rodents and humans</td>
</tr>
<tr>
<td>2006</td>
<td>Gerald I Shulman</td>
<td>Role of dysregulated intracellular lipid metabolism in insulin resistance</td>
</tr>
</tbody>
</table>
On July 30, 2012, Dr. George F. Cahill, Jr. passed away in Peterborough, New Hampshire at the age of 85. Dr. Cahill was a world-renowned diabetes researcher who served as Joslin’s Research Director from 1962 to 1978.

Dr. Cahill did his undergraduate education at Yale (which he described as “one party with a few academics thrown in”) and then went to Columbia College of Physicians and Surgeons, where his father was on the faculty as a urologic surgeon. After medical school, George came to the Peter Bent Brigham Hospital in Boston, where George W. Thorn, the great endocrinologist, was Physician-in-Chief. He was strongly influenced by Thorn, as well as by his senior resident, a Swiss physician named Albert Renold, who also was destined to become a great diabetes researcher. Renold had spent two years in the Department of Biological Chemistry with Prof. A. Baird Hastings prior to his Brigham senior residency studying carbohydrate metabolism and, following this example, after his second clinical year, Cahill joined the Hastings laboratory to study glucose metabolism. After two years in the Hastings lab, he returned to the Brigham for another clinical year and then joined Renold, who had moved to the Baker laboratories at the New England Deaconess Hospital. In 1962, when Renold returned to Switzerland, Cahill took the reins as head of research as the Baker labs joined the Joslin Foundation and began to evolve into what is now the Research Division of the Joslin Diabetes Center. Cahill also served as head of the Endocrine-Metabolic Unit of the Brigham and gradually rose up the academic ranks to become Professor of Medicine at Harvard Medical School.
Cahill’s research at Joslin focused on defining the normal physiology of glucose and amino acid homeostasis during feeding and fasting, as well as in obesity and diabetes. His studies set forth many of the tenets that form the basis of our classic understanding of these processes. His early interest in ketoacid metabolism stayed with him through his life, and even led to a late interest in developing high energy supplements for military personnel in combat areas situations on this pathway.

Cahill was also a devoted and supportive mentor who trained many of the individuals who went on to further the field, including Oliver Owen, Philip Felig, Errol Marliss, Thomas Aoki, Guillermo Herrera, Neil Ruderman, Aldo Rossini, Fred Morgan and Murray Brennan. As a result, Cahill’s influence on diabetes research was felt worldwide through both his many seminal discoveries and through the training of hundreds of fellows and students who have become leaders in diabetes research, care and education throughout the world. In addition, for those of us who knew him, Dr. Cahill’s skill, unique style and passion for teaching of students, young investigators and colleagues of all ages is one of the hallmarks of his remarkable career at Joslin, the Brigham and Women’s Hospital, the New England Deaconess Hospital, and Harvard Medical School.

In 1972, George joined the Scientific Advisory Board of Howard Hughes Medical Institute (HHMI), which was in control of candidate selection, as well as reviews of investigator performance. In 1978, he left Joslin to become Director of Research for HHMI where his influence on this organization increased. He was eventually elevated to a Vice President of HHMI. In 1989, he resigned that position to move to his home in Stoddard, NH. Being the consummate teacher, he joined the faculty of Dartmouth College (about 50 miles away) to support their biology programs and was given the title of Professor of Biological Sciences. There, he taught a course of biology for non-scientists which was so popular that within a few days after starting, they had to move from a classroom designed for 100 to an auditorium that held more than 400 students. He taught this course for seven years and received a teaching award from the students who loved it. In 1996 he retired completely to spend more time with his wife Sally, his children and grandchildren.

In addition to his many academic skills George was fit and trim throughout his life, and a great athlete, being an extremely competitive squash and tennis player. He was always intellectually engaged, but also thought broadly in science, outside his own area of metabolic expertise. His career was recognized by many honors and awards, including a symposium held in his honor at Joslin in November, 2006. George Cahill was a very important man in the history of diabetes research of the 20th century. Both the Joslin and the world will miss him.

In 2006 the George Cahill, M.D. Scholarship Fund was created to provide a permanent source of funding for student positions at Joslin during the summer, continuing Dr. Cahill’s great tradition of mentoring young investigators just beginning their careers in diabetes research.
In the memory of George F. Cahill Jr., the Montreal Diabetes Research Center is proud to organize each year “The George F. Cahill Jr. lecture” given by world-leaders and outstanding speakers.

The George F. Cahill Jr Lecture 2017 will be given on February 3 at the CRCHUM by Dr. David M Nathan from Harvard Medical School, Boston, MA. His lecture is entitled: *On diapers and septic fields: recent Advances in the prevention and Treatment of Type 2 Diabetes.*

Previous George F. Cahill Jr lecturers were:

<table>
<thead>
<tr>
<th>Year</th>
<th>Recipient</th>
<th>Title of the lecture</th>
</tr>
</thead>
<tbody>
<tr>
<td>2018</td>
<td>Jean-Pierre Després</td>
<td>Obesity, lifestyle and cardiometabolic diseases: time to align clinical practice/public health recommendations to scientific evidence</td>
</tr>
<tr>
<td>2017</td>
<td>David M Nathan</td>
<td>On diapers and septic fields: recent Advances in the prevention and Treatment of Type 2 Diabetes.</td>
</tr>
<tr>
<td>2016</td>
<td>Daniel J Drucker</td>
<td>Redefining classical concepts of incretin hormone action.</td>
</tr>
<tr>
<td>2015</td>
<td>Ralph DeFronzo</td>
<td>Treatment of Type 2 Diabetes: A rational approach based on its pathophysiology.</td>
</tr>
<tr>
<td>2014</td>
<td>Bernard Zinman</td>
<td>The Diabetes Control and Complications Trial (DCCT). Impact on our understanding and prevention of Complications in Type 1 DM.</td>
</tr>
</tbody>
</table>
1 - A link between early-life exposure to environmental pollutants and diabetes risk

Myriam Hoyeck¹, Jenny Bruin¹

¹Carleton University

Diabetes prevalence is increasing at exponential rates, and epidemiological studies have shown a correlation between pollutant exposure and diabetes incidence. Dioxins are a group of highly persistent organic pollutants that show widespread global distribution. Preliminary data in the Bruin lab has shown that exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most toxic dioxin, upregulates Cyp1a1 expression (a biomarker of dioxin exposure) in islets, suppresses glucose-stimulated insulin secretion in human and mouse adult β-cells, and causes β-cell loss in adult mice. Abnormalities in β-cell mass and function are key characteristics of diabetes, as such TCDD exposure may pre-dispose females to diabetes-associated complications during pregnancy. In addition, studies have shown that dioxins cross the placenta and are excreted in breast milk; therefore, it is possible that maternal exposure to dioxins could alter pancreas development and function in offspring and may confer a lifetime risk of developing diabetes. This study analyzed the effects of chronic low-dose TCDD exposure to dams during gestation and lactation on pancreas development and long-term diabetes risk in both male and female offspring, as well as effects on dam metabolism. Female mice were treated with TCDD (20 ng/kg/d) or corn oil (vehicle) 2x per week prior to and throughout gestation, and during lactation until weaning. Plasma, liver, and pancreas were collected from offspring at birth and weaning (3 weeks of age) for analysis by ELISA, qPCR, and immunohistochemical staining. A subset of offspring was transferred to a high fat diet (HFD) at 12 weeks of age (9 weeks after TCDD exposure ceased), and a subset of dams received HFD beginning at 8 weeks post-exposure. Long-term changes in pancreas function were assessed pre- and post-HFD using in vivo glucose-stimulated insulin secretion assays, and glucose and insulin tolerance tests. Neonates from TCDD-treated dams had significantly decreased blood glucose levels and increased plasma insulin levels compared to control offspring at birth. The decrease in blood glucose persisted until 3 weeks. At 6-9 weeks, TCDD-exposed males were modestly hyperglycemic during a glucose tolerance test and significantly insulin resistant, whereas female offspring did not display lasting effects on glucose homeostasis. Interestingly, TCDD exposure during pregnancy did not have lasting effects on blood glucose or insulin secretion in the dams, but did promote increased weight gain relative to control dams starting approximately 5 weeks post-exposure. The effect of HFD on TCDD-exposed offspring and dam pancreas function is currently being assessed. These results suggest that early-life exposure to TCDD may predispose male offspring to defects in pancreas function and increased diabetes risk. Additionally, TCDD exposure during pregnancy promotes excessive weight gain in dams. Taken together, our data supports epidemiological evidence that pollutant exposure may be a causal factor driving diabetes risk.
2 - 14-3-3zeta is required for PKA-dependent lipolysis

Abel Oppong, Yves Mugabo, Gareth Lim

CRCHUM/ Université de Montréal

The molecular scaffold, 14-3-3z, was previously found to be essential for visceral adipogenesis, but its contributions to the function of mature adipocytes is not known. As it can regulate the activities of metabolic effectors, we hypothesized that 14-3-3z also has essential roles in adipocyte function.

3T3-L1 adipocytes and mouse models were used to study if 14-3-3ζ regulates lipolysis. Depletion of 14-3-3ζ by siRNA abrogated glycerol and free fatty acid (FFA) release from 3T3-L1 cells treated with Isoproterenol (ISO, 1 μM), Forskolin (FSK, 10 μM), and dibutyryl cAMP (1 mM). In contrast, over-expression of 14-3-3ζ potentiated ISO-mediated FFA release. Knockdown of 14-3-3ζ did not affect cAMP generation in ISO- and FSK-treated 3T3-L1 cells, but mRNA levels of lipases (Atgl, Hsl, and Magl) and Pparg were reduced, suggesting a loss of adipocyte identity. Decreased activation and total expression of PKA substrates, including Hsl and CREB, were detected in 14-3-3ζ-depleted 3T3-L1 cells. Taken together, these data suggest that 14-3-3ζ is necessary for lipolysis from 3T3-L1 adipocytes.

To understand adipocyte-specific roles of 14-3-3ζ, tamoxifen (TMX)-inducible, adipocyte-specific 14-3-3ζ knockout (adi14-3-3zKO) mice were used. Four weeks after TMX exposure (5 days, 50 mg/kg), no effects on body weight were found. After an overnight fast, adi14-3-3zKO mice displayed impaired lipolysis following i.p CL-316,243 (1 mg/kg) injections. In contrast, transgenic over-expression of 14-3-3ζ did not affect lipolysis. Adi14-3-3zKO mice also displayed glucose intolerance following i.p. glucose (2 g/kg). Real-time PCR confirmed significant reductions in Atgl, Hsl, and Pparg mRNA levels in adi14-3-3zKO mice, suggesting impaired adipocyte function.

Collectively, these results demonstrate essential functions of 14-3-3ζ in facilitating lipolysis and, potentially, adipocyte maturity. Future studies are aimed at understanding how 14-3-3ζ regulates other aspects of adipocyte function, including diet-induced expansion of fat mass.
3 - Omega-3 fatty acids inhibit the interleukin-1β pathway in white adipose tissue and correlate with an improvement in C-peptide secretion in humans

Valérie Lamantia1,2, Simon Bissonnette1,2, Yannick Cyr1,2, Viviane Provost3, Marie Devaux2, May Faraj1,2

1Université de Montréal, 2Institut de recherches cliniques de Montréal, 3Centre hospitalier de l'Université de Montréal

Objective: Activation of the interleukin-1β (IL-1β) pathway induced by the NLRP3 inflammasome promotes white adipose tissue dysfunction (WAT) and type 2 diabetes (T2D) in humans. Omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), ameliorate insulin resistance in humans and inhibit IL-1β secretion in murine WAT, but their effect on the IL-1β pathway in human remains unclear. We tested the hypothesis that EPA and DHA inhibit the IL-1β pathway in human WAT in vivo and ex vivo.

Methods: We examined 33 non-diabetic subjects (45-74 years, >20 kg/m², 64% postmenopausal women) before and after 12-week supplementation of 1.6 g EPA and 1.1 g DHA/day. Glucose-induced insulin and C-peptide secretions and insulin sensitivity were measured during a 3h-Botnia clamp. Fasting red blood cell (RBC) phospholipid EPA and DHA were measured by gas chromatography mass spectrometry. Protein expression of NLRP3, pro-IL-1B and procaspase-1 were measured in fasting WAT by immunoblot. The IL-1β secretion from fasting WAT was measured by alpha-LISA. Direct effect of EPA+DHA (200 mmol/L) on fasting WAT IL-1β secretion was examined over 7 hours following WAT stimulation with lipopolysaccharide (LPS) and adenosine triphosphate (ATP) (positive controls for maximum secretion) or native LDL (metabolic signals).

Results: At baseline, %EPA+DHA in RBC phospholipids was associated negatively with WAT IL-1β secretion (r=-0.46, p=0.029). There was a significant increase in %EPA+DHA in RBC phospholipids (baseline=1.62±0.74% vs post-intervention=3.75±1.93%, p<0.001). Best responders to EPA:DHA to inhibit WAT NLRP3 inflammasome activity were subjects with higher baseline activity, as post-intervention changes in pro-IL-1β, procaspase-1 and IL-1β secretion were inversely correlated to their baseline levels (r=-0.64, r=-0.63 and r=-0.72 respectively, p<0.01). Moreover, post-intervention change in WAT IL-1β secretion correlated positively with that of C-peptide secretion (r=0.58, p=0.048). Addition of EPA+DHA inhibited IL-1β secretion by LPS+ATP-stimulated (-88.6%, p <0.05) or LDL-stimulated WAT (-81.4%, p<0.01).

Conclusion: The improvement in C-peptide secretion following EPA and DHA supplementation is associated with the amelioration in WAT IL-1β pathway. Omega-3 supplementation may aid in the prevention of T2D in subjects with activated WAT IL-1β pathway.
4 - The role of PGC-1α in NAFLD-associated liver-cancer.

Mélissa Léveillé¹, Aurèle Besse-Patin¹, Flavie Marquis², Cindy Baldwin¹,², Bich Nguyen³, Jennifer Estall¹

¹IRCM, ²McGill University, ³CHUM

INTRODUCTION: Nonalcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver biochemistry in North America. It is now estimated that 10-22% of hepatocellular carcinoma (HCC) cases are attributed to fatty liver disease. However, the mechanisms underlying the relationship between diet-related hepatic metabolic disease and cancer development are poorly understood. PGC-1α is a master regulator of metabolism. Interestingly, low levels of PGC-1α are reported in patients diagnosed with inflammatory fatty liver disease and human HCC. We aim to determine whether low hepatic PGC-1α, when combined with a western diet, potentiates the development of liver cancer.

METHODS: We developed a mouse model of NAFLD-associated liver cancer by combining a high-fat/high-fructose diet (HFHFD) with a "second hit" of diethylnitrosamine (DEN). Female and male mice expressing either one (LH, liver heterozygotes) or two (LKO, liver knockouts) floxed PGC-1α alleles under the control of the albumin promoter were subjected to the protocol. Weights were monitored weekly and serum samples taken bi-monthly. Body composition, liver tumour multiplicity and maximum size were quantified. RNA and protein samples from adjacent liver tissues and tumours were used to investigate pathways known to influence nutrient metabolism, oxidative stress and hepatocarcinogenesis.

RESULTS: We observed a decrease in PGC-1α expression in liver tumours of mice given the carcinogen (DEN) in combination with the high-fat/high-fructose diet. We also observed a similar trend in human HCC. After quantifying liver tumour multiplicity and maximum size in whole livers of mice subjected to NAFLD-induced HCC protocol, our data shows that LKO male mice exhibit increased liver tumour number and maximum size. Interestingly, loss of hepatic PGC-1α significantly reduces oxidative metabolism, G1-phase marker yH2AX and apoptotic marker cleaved-caspase 3. This reduction is associated with a significant increase in p-ERK and p-STAT3 in adjacent liver tissues. Also, tumours from LKO male mice exhibit upregulated B-catenin/TCF target genes (Cyclin D1, MYC, CD44, SOX9). Finally, gain- and loss-of-function experiments show that PGC-1α regulates E-cadherin gene expression and B-catenin subcellular localization.

In conclusion, loss of hepatic PGC-1α combined with an obesogenic diet promotes NAFLD-associated liver cancer. Moreover, loss of hepatic PGC-1α may impact the G1 checkpoint entry, cell survival and cancer stem cell potential, in part, through differential B-catenin subcellular localization. Our data implicates PGC-1α as an important mitigating factor in the development of diet- and NAFLD-associated liver cancer.
5 - HB-EGF signaling is required for glucose-induced pancreatic β-cell proliferation in rats

Hasna Maachi1,2,3, Mallikarjuna R. Metukuri4, Donald Scott4, Julien Ghislain1,2, Vincent Poitout1,2,3

1Montreal Diabetes Research Center, 2CRCHUM, 3Department of Medicine, University of Montreal, 4Icahn School of Medicine at Mount Sinai, NY, USA

Background: Glucose is a major β-cell mitogen. Despite recent progress, the underlying mechanisms remain unclear. In a rat model of nutrient excess we previously showed that nutrient-induced β-cell proliferation is blocked when either EGF receptor (EGFR) or mTOR signalling is inhibited. Parallel transcriptomic analyses identified the EGFR ligand, HB-EGF as a potential mediator of nutrient-induced β-cell proliferation.

Objective: To determine the role of HB-EGF in glucose-induced β-cell proliferation.

Methods: HB-EGF mRNA levels were assessed by real-time PCR in isolated rat islets following a 24-h exposure to 2.8 or 16.7 mM glucose. The Carbohydrate-Responsive Element-Binding Protein (ChREBP) transcription factor was down-regulated by siRNA in dispersed rat islets. For β-cell proliferation studies islets were exposed to 16.7 mM glucose or HB-EGF (100 ng/ml) in the presence of 2.8 mM glucose for 72 h. Islets were co-cultured with the EGFR inhibitor AG1478 (300 nM) or the HB-EGF inhibitor CRM197 (10 ug/ml). shRNA was used to knockdown HB-EGF in isolated islets and either cultured ex vivo or transplanted under the kidney capsule of glucose-infused rats. β-cell proliferation was assessed by immunohistochemistry for Ki67 and insulin.

Results: Glucose increased HB-EGF mRNA levels and this was prevented by ChREBP knockdown. HB-EGF potently stimulated β-cell proliferation. Inhibition of the EGFR or HB-EGF completely blocked not only the proliferative response to HB-EGF but also the response to 16.7 mM glucose. Knockdown of HB-EGF blocked the β-cell proliferative response to glucose in isolated rat islets as well as in transplanted islets.

Conclusion: HB-EGF is a potent β-cell mitogen in rat islets. Glucose increases HB-EGF gene expression via ChREBP. The proliferative response to glucose requires an intact HB-EGF – EGFR pathway. Our findings identify a novel player in the complex mechanisms controlling β-cell proliferation in response to glucose. (NIH, FRQS)
6 - Genetic disruption of Adipose Triglycerides Lipase (ATGL) in mediobasal hypothalamic neurons induces overweight and metabolic disturbances

Romane Manceau1,2, Sébastien Audet1,2, Arturo Israel Machuca-Parra1, KHALIL BOUYAKDAN1,2, Demetra Rodaros1, Alexandre Fisette2, Grant Mitchell3, Stephanie Fulton1,2, Thierry Alquier1,2

1CRCHUM, 2Université de Montréal, 3Ste-Justine Hospital

Background: Adipose Triglyceride Lipase (ATGL) acts as the first lipase in the hydrolysis of triglycerides (TG). Recent studies show that ATGL in peripheral tissues plays major roles on energy homeostasis. We found that ATGL is expressed in the mediobasal hypothalamus (MBH) and in hypothalamic neuronal cell lines, in line with our recent study suggesting that neurons accumulate TG. ATGL expression is increased in the MBH of high fat-fed mice that maintain a healthy body weight compared to mice that become obese. In addition, ATGL expression in the MBH is increased in response to fasting. This suggests that increased ATGL may play a role in maintaining a healthy metabolic profile. We propose that hypothalamic ATGL regulates lipid metabolism in the brain that in turn contributes to energy balance.

Materials and methods: To test this hypothesis, synapsin-Cre or -GFP expressing AAV are stereotaxically injected in the arcuate nucleus (ARC) of male ATGL flox mice to KO ATGL specifically in neurons (ATGLKO).

Results: First, we validated that ATGL expression is reduced by 50% in ATGLKO mice. We found that ATGLKO have increased weight gain on a chow diet compared to control animals that is associated with reduced energy expenditure and increased food intake and fat mass. In addition, chow-fed ATGLKO mice have an increased fasting glycaemia and mild glucose intolerance. Finally, pharmacological inhibition of ATGL in hypothalamic neurons in vitro increases intracellular TG content.

Conclusion: Together, our findings suggest that the ATGL pathway in MBH neurons beneficially regulates glucose and energy homeostasis by mechanisms that may involve regulation of TG and lipid droplets metabolism.
Vitamin K is the cofactor of γ-carboxylase an enzyme that convert the glutamic acid residues into γ-carboxyglutamic acid residues in some secreted proteins. Clinical data suggest that vitamin K may influence the development of diabetes, but the γ-carboxylated protein(s) and the mechanisms involved remain unknown. Growth arrest-specific 6 (GAS6) is a secreted γ-carboxylated protein that acts as a ligand for the TAM tyrosine kinase receptor family, which includes, TYRO3, AXL, and MERTK. GAS6 and its receptors play a critical role in the immune system, in tumor progression, and in cancer metastasis. More recent studies found that circulating GAS6 levels or SNPs in Gas6 gene are associated with obesity and insulin resistance in humans, however, the mechanism by which GAS6 influences these metabolic disorders is not understood.

We therefore decided to 1) investigate the role of GAS6 in the development of insulin resistance and diabetes in vivo using mouse models; and 2) determine the signalling pathways involved.

We first noticed in wild-type mice that the serum levels of GAS6 are higher in feeding condition compared to fasting, suggesting that the secretion of this factor is regulated by the nutritional and/or the energetic status of the animals. Next we characterize the function of GAS6 in energy metabolism, by analyzing the metabolic phenotype of 3-month-old Gas6−/− male mice fed a normal diet. We found that these mice have improved glucose tolerance and insulin sensitivity, but normal insulin secretion, when fed a normal chow diet. This phenotype was also observed at 16 months of age and in young mice fed a high-fat diet, suggesting that the absence of GAS6 protects from age- or diet-induced insulin resistance. Conversely, in a transgenic gain-of-function model (i.e., ApoE-Gas6Tg), increased GAS6 circulating levels is sufficient to cause reduced insulin sensitivity.

TAM receptors gene expression analysis in insulin-sensitive tissues revealed that AXL is highly expressed in skeletal muscle and white adipose tissue. We further show that AXL is activated by GAS6 in myotubes (C2C12) and adipocytes (3T3-L1) in culture. We therefor characterized the GAS6/AXL-dependent transcriptome in C2C12 myotubes following treatment with recombinant GAS6 or a pharmacological inhibitor of TAM receptors (LDC1267). Interestingly, RNA sequencing analysis revealed that GAS6/AXL signalling regulates the expression of genes encoding proteins involved in signalling downstream of the insulin receptor (e.g., Irs2 and Eif4ebp1) and in cholesterol biosynthesis (e.g., Hmgcs1, Hmgrc, Nsdhl, Fdft1, etc.). We further show that several of these genes are up-regulated in the skeletal muscle of Gas6−/− mice at 16 months of age. In addition, we found a decrease in the cholesterol levels of myotubes treated with GAS6 (200 ng/ml) in vitro. In the presence of a pharmacological inhibitor of AXL (R428), the effect of GAS6 is blunted, suggesting that GAS6 inhibits cholesterol synthesis in an AXL-dependent manner in muscle cells.

Our results suggest that GAS6, via its receptor AXL, may reduce insulin sensitivity by inhibiting the insulin signalling pathway. GAS6 also inhibits cholesterol biosynthesis in muscle cells. Since cholesterol is synthesized from Acetyl-CoA, a product of glycolysis, we propose that GAS6, by decreasing the expression of cholesterol biosynthesis genes, decreases cholesterol formation, inducing a decrease in glucose consumption by the muscle. Altogether, these effect of GAS6 on muscle, could favour hyperglycemia and the development of diabetes.
8 - High Fat Diet Leads to Alterations of Hepatic Lipid and Amyloid β Metabolism in the 3xTg-AD Mouse Model of Alzheimer’s Disease

Cristina R. Bosoi1,2,4, Milene Vanda3,4, Marine Tournissac3,4, Hortense Fanet3,4, Jessica Virgili3,4, Robert Lippman5, Jasmohan S. Bajaj6, Andre Marette1,2, Frederic Calon3,4

1Centre De Recherche De L’institut De Cardiologie Et Pneumologie De Québec, 2Faculté De Médecine, Université Laval, 3Faculté De Pharmacie, Université Laval, 4Axe Neurosciences, Centre De Recherche Du CHU-Q (Pavillon CHUL), 5Mcguire VA Medical Center, VA, E-U, 6Virginia Commonwealth University, VA, E-U

Background: Fatty liver as well as cognitive and memory alterations are both known complications of obesity and type II diabetes. Alzheimer’s disease (AD), the most common cause of dementia, is increasingly considered as a peripheral metabolic disease. The presence of type II diabetes and obesity in midlife are well-known risk factors for developing AD at an older age. The relationship between AD and the liver has not been investigated yet. However, several liver-derived circulating lipid species have been proposed as biomarkers of AD. Amyloid β (Aβ) is a peptide with an unclear physiological function, which forms the amyloid plaques found in brains of AD patients and which is released in the circulation. The liver is an important Aβ clearing organ, yet its role during the disease remains unknown. The 3xTg-AD mouse model reproduces both Aβ and tau pathologies characteristic to AD neuropathology, but also peripheral metabolic impairments such as glucose intolerance. Since the liver plays a major role both in maintaining glucose and lipid homeostasis as well as in Aβ clearance, our aim was to explore the hepatic contribution to the peripheral metabolic alterations present in 3xTg-AD mice.

Methods: 3xTg-AD mice and non-transgenic mice on the same background (NonTg) received a high fat (HFD) or a control diet (CD) for 9 months and were sacrificed at 15 months of age. We assessed hepatic histology by hematoxilin-eozin staining and lipid content with commercial kits following chloroform–methanol extraction. Liver enzymes involved in lipid metabolism, gluconeogenesis and Aβ clearance were measured by qPCR and Western blot.

Results: Liver weight, hepatic lipid content and steatosis scores were not changed between NonTg and 3xTg-AD mice. Interestingly, these parameters increased following HFD only in NonTg, but not in 3xTg-AD mice. HFD decreased the phosphorylation level of liver acetyl-CoA carboxylase as well as expression of fatty acid synthase in NonTg and even more strikingly in 3xTg-AD mice (obese 3xTg-AD: p<0.05 vs obese NonTg for both enzymes). Expression of sterol regulatory element-binding protein 1 increased only in obese 3xTg-AD (p<0.05 vs all other groups). PPARα expression was decreased in HFD-fed NonTg mice and remained similar between obese 3xTg-AD and non-obese controls (obese 3xTg-AD: p<0.05 vs obese NonTg; p>0.05 vs NonTg). Gluconeogenic enzymes Pck1 and G6pc were not changed. Circulating Aβ and its hepatic receptor LRP1 were not changed between 3xTg-AD groups. However, expression of hepatic neprrylisin, the main enzyme involved in the clearance of Aβ, significantly decreased in obese 3xTg-AD mice compared to all other groups.

Conclusion: Our results indicate that hepatic lipid accumulation is prevented in obese 3xTg-AD mice due to increased hepatic fatty acid oxidation and decreased lipogenesis. Hepatic clearance of Aβ is impaired by obesity in 3x-Tg-AD mice. Our results highlight the importance of peripheral metabolism in the pathogenesis of AD. A better comprehension of the mechanisms relating AD and peripheral metabolism may uncover potential therapeutic targets.
Lack of Tubular Heterogeneous Nuclear Ribonucleoprotein F (hnRNP F) Attenuates Kidney Hypertrophy and Glomerular Hyperfiltration in Diabetic Akita mice

Kana N. Miyata1, Chao-Sheng Lo1, Shuiling Zhao1, Isabelle Chenier1, Janos G. Filep2, Julie R. Ingelfinger3, Shao-Ling Zhang1, John S. D. Chan1

1CRCHUM, Université de Montréal, Montreal, QC, Canada, 2Research Centre, Maisonneuve-Rosemont Hosp., Montreal, QC, Canada, 3Pediatric Nephrology Unit, Mass. Gen. Hosp., Boston, MA, United States

Background

Kidney hypertrophy and glomerular hyperfiltration are known to precede the development of proteinuria and reduced renal function in patients with diabetes. The amelioration of glomerular pressure has been proposed as an underlying mechanism of the improved renal outcome in recent clinical studies with sodium-glucose co-transporter-2 (SGLT2) inhibitors, a new category of oral anti-diabetic agents.

We previously reported that a deficiency of heterogeneous nuclear ribonucleoprotein F (hnRNP F) in renal tubules down-regulates SGLT2 in non-diabetic mice (ASN Abstract, 2018). Non-diabetic tubule-specific hnRNP F knockout (KO) mice did not demonstrate any significant difference in blood glucose, kidney weight/body weight (KW/BW) ratio, or glomerular filtration rate (GFR) compared to control mice.

Objective

To investigate the impact of hnRNP F deficiency in diabetic mice at hyperfiltration stage.

Methods

Tubule-specific hnRNP F KO mice were generated as described previously via cross-breeding of Pax8-Cre mice with floxed hnRNP F mice on a C57BL/6 background. Akita-hnRNP F KO mice were created by cross-breeding of female tubule-specific hnRNP F KO mice with male heterozygous Akita mice. Male adult (8 weeks of age) Akita-hnRNP F KO mice, Akita mice, and control wild-type (WT) littermates were studied (n=4 per group). Blood glucose was measured by glucometer up to 24 weeks of age. At 24 weeks, GFR was measured by inulin-FITC clearance in awake mice prior to euthanization, and kidneys were processed for histology. The results are expressed as the mean ± SEM.

Results

Akita-hnRNP F KO mice had consistently lower blood glucose levels than Akita mice (WT 9.18±0.24 vs Akita 34.0 vs Akita-hnRNP F KO 28.0±2.69 at 24 weeks; p<0.01). Akita-hnRNP F KO mice had lower KW/BW ratio than Akita mice (WT 0.85±0.06 vs Akita 2.10±0.21 vs Akita-hnRNP F KO 1.44 ±0.13; p<0.01). In addition, GFR/BW ratio tended to be lower in Akita-hnRNP F KO mice than Akita mice (WT 6.23±0.19 vs Akita 17.40±5.48 vs Akita-hnRNP F KO 10.45±1.32). By histology, no significant signs of renal injury were observed in either of the groups. However, kidney tissues showed attenuated glomerulomegaly and decreased number of SGLT2-positive tubules in Akita-hnRNP F KO mice compared to Akita mice.

Conclusions

Our data demonstrate that kidney hypertrophy was attenuated in Akita-hnRNP F KO mice, likely by down-regulated SGLT2 lowering blood glucose levels as well as activating tubuloglomerular feedback, which decreases single nephron GFR. SGLT2 inhibitors are garnering attention for their renoprotective effects in diabetics. Our data indicate that Akita-hnRNP F KO mice can act as a unique preclinical tool to study the physiological effects of SGLT2 down-regulation.
Adipose α/β-hydrolase domain-6 is a negative modulator of adipose thermogenesis and its inhibition promotes metabolically healthy obesity

Pegah Poursharifi\textsuperscript{1}, Camille Attané\textsuperscript{1}, Yves Mugabo\textsuperscript{1}, Anfal Almass\textsuperscript{1,2}, Shangang Zhao\textsuperscript{1}, Roxane Lussier\textsuperscript{1}, Heidi Erb\textsuperscript{1}, Julian Guida\textsuperscript{1}, Elite Possik\textsuperscript{1}, Marie-Line Peyot\textsuperscript{1}, Erik Joly\textsuperscript{1}, Andre Tchernof\textsuperscript{3}, Christophe Noll\textsuperscript{4}, Andre C. Carpentier\textsuperscript{4}, S.R. Murthy Madiraju\textsuperscript{1}, Marc Prentki\textsuperscript{1}

\textsuperscript{1}CRCHUM, \textsuperscript{2}McGill University, \textsuperscript{3}Université Laval, \textsuperscript{4}Université de Sherbrooke

Obesity is a rapidly growing threat to the global health and activation of energy expenditure processes in the brown adipose tissue (BAT) and white adipose tissue (WAT) may provide solutions. Pan-deletion of the lipase alpha/beta-domain hydrolase-6 (ABHD6) demonstrated the therapeutic potential of ABHD6 inhibitors against obesity and type-2-diabetes; though the precise depot-specific role of ABHD6 in the adipose tissue metabolism remains unexplored.

ABHD6\textsuperscript{floxflo} mice were bred with Adipoq-Cre/ERT2 mice and Abhd6\textsuperscript{floxflo}/Adipoq-Cre to obtain AT-ABHD6-KO mice by tamoxifen injection. Control littermates and KO mice were fed a normal diet (ND) or a high fat diet (HFD) for 12 weeks. For thermogenesis studies, ND mice were kept either at room temperature or exposed to cold temperature (4°C and 10°C) for 24h - 48h.

Our results show that adipose tissue ABHD6 expression level increases during adipocyte differentiation, and correlates with adiposity in WAT from HFD mice and in visceral fat depot from patients with obesity. AT-ABHD6-KO mice on ND showed similar phenotype as controls at room temperature, but were resistant to cold induced hypothermia and displayed reduced adipocyte size and inflammation. The enhanced energy expenditure in cold was in part due to accelerated glycerolipid/fatty acid futile cycle in visceral adipose and oxidative metabolism in BAT. Under cold-exposure 2-monoacylglycerol (MAG) levels were increased in the WAT from KO mice. The mRNA expression of PPAR alpha/PPAR gamma target genes were induced by 2-MAG treatment. In addition, we found that 2-MAG is also capable of activating PPAR alpha in transactivation studies, suggesting that PPAR activation by accumulating 2-MAG contributed to thermogenic mechanisms in the cold exposed AT-ABHD6-KO mice. KO mice on HFD displayed healthy-obese characteristics, including improved insulin sensitivity, elevated WAT beta-oxidation, lower liver TG content, and improved systemic and WAT inflammation.

The results indicate that ABHD6 negatively modulates the adipose thermogenic program and AT-ABHD6 deletion protects from cold induced hypothermia and also promotes a healthy-obese phenotype in HFD fed mice.
**Poster Index**

A- Drug development / Devices / Genetics / Human Studies

1 - Alginate Beads with Superparamagnetic Iron Oxide Nanoparticles for Pancreatic Islets Encapsulation and Imaging.
John Jahchan

2 - Caractérisation in vivo d’un nouveau lien unissant le stress oncogénique à la régulation du cycle cellulaire.
Audrey Poirier

3 - Regulation of glucocorticoid receptor turnover by USP19 deubiquitinating enzyme
Erin Coyne

4 - ZNF768 connecte la signalisation des facteurs de croissance au contrôle de la prolifération.
Romain Villot

B- Obesity / Adipose Tissue studies / Insulin Resistance

5 - 14-3-3zeta is required for PKA-dependent lipolysis
Abel Oppong

6 - Adipose α/β-hydrolase domain-6 is a negative modulator of adipose thermogenesis and its inhibition promotes metabolically healthy obesity
Pegah Poursharifi

7 - Development of a method to identify new regulatory processes controlling GLUT4 translocation
Nolwenn Samson

8 - Effet de l'absence de la protéine IGFBP-2 sur le profile lipidique et le développement de la plaque d'athérome chez des souris femelles.
Chloé Rauzier

9 - Identification d'une nouvelle cible de PCSK9: Dégradation du récepteur de l'insuline dans le foie de modèles murins par PCSK9
Julie Cruanes
10 - Modulation de la production hépatique d'IGFBP-2 par FGF19.
Justine Faramia

11 - Normocholesterolemic subjects with lower plasma PCSK9 display white adipose tissue dysfunction and activated IL-1beta system
Yannick Cyr

12 - Neurobehavioral correlates of obesity are largely heritable
Uku Vainik

13 - Modulation of the insulin-like growth factor-1 axis after bariatric surgery in morbidly obese patients
Meng Li

14 - Omega-3 fatty acids inhibit the interleukin-1β pathway in white adipose tissue and correlate with an improvement in C-peptide secretion in humans
Valérie Lamantia

15 - Risk of cardiometabolic complications in cystic fibrosis patients who are overweight or obese and those who go through a significant weight change
Anne Bonhoure

16 - Role of GAS6 in insulin sensitivity and cholesterol biosynthesis
Céline Schott

17 - The Role of 14-3-3ζ in the “beiging” of white adipose tissue
Kadidia Diallo

18 - Vitamin K in adults with cystic fibrosis is correlated to fat mass and insulin secretion
Johann Colomba
C- Neurobiology and energy homeostasis / Exercise

19 - Cibler le domaine alpha / bêta-hydrolase 6 dans le contrôle neuronal de l'alimentation et de la récompense
David Lau

20 - Comparaison de deux stratégies d'apport en glucides pour améliorer le contrôle de la glycémie pendant l'exercice chez les adolescents et adultes atteints de diabète de type 1 (résultats préliminaires)
Lucas Goulet Gélinas

21 - Genetic disruption of Adipose Triglycerides Lipase (ATGL) in mediobasal hypothalamic neurons induces overweight and metabolic disturbances
Romane Manceau

22 - Leptin receptor expression and the blood brain barrier (BBB)
Liliia Butiaeva

23 - Inhibition of ATGL reduces inflammation in LPS-activated microglial cells
Arturo Israel Machuca-Parra

24 - Novel glycerol-3-phosphate phosphatase/PGP-like homologs are implicated in metabolism, stress responses and lifespan in C. elegans.
Elite Possik

D- β-Cell, Liver and Bone Biology

25 - A link between early-life exposure to environmental pollutants and diabetes risk
Myriam Hoyeck

26 - Beta-cell compensation to pubertal insulin resistance is compromised in high-fat fed rats and impairs glucose homeostasis later in life
Anne-Laure Castell

27 - BMP9 (Bone Morphogenetic Protein-9) non-canonical signaling Akt/Fox01 inhibits hepatic gluconeogenesis via Alk3
Naoufal Akla

28 - Defining the cellular mechanisms of DPP4 shedding
Branka Vulesevic
29 - Defining the short-term effects of pharmacological 5’-AMP activated kinase modulators on kidney cell mitochondria
Dana Abou Samhadaneh

30 - Deletion of 14-3-3ζ in pancreatic β-cells potentiates glucose-stimulated insulin secretion
Yves Mugabo

31 - Etude de l’interaction de Nck1 et PERK dans la fonction et la survie des cellules beta pancréatiques
Emilie Courty

32 - HB-EGF signaling is required for glucose-induced pancreatic β-cell proliferation in rats
Hasna Maachi

33 - Identification de facteurs sécrétoires dépendants de l’expression hépatique de PGC-1a dans un contexte de développement de la stéatose hépatique non alcoolique.
Philipa Levesque-Damphousse

34 - Identification of a new hepatokine expressed and secreted in response to steatosis.
Mathilde Mouchiroud

35 - PGC-1α4 attenuates apoptosis in response to an inflammatory challenge
Stewart Jeromson

36 - Role of de novo sphingolipid metabolites in oleate-induced pancreatic β-cell proliferation in rats
Alexis Vivoli

37 - Stability of the bone-derived hormone osteocalcin is regulated through its O-glycosylation in mice, but not in human
Omar Al Rifai

38 - TCDD impairs differentiation toward human pancreatic beta cell fate in vitro
Erin MacFarlane
E- Diabetes Complications, CVD, Microbiota and T1D studies

39 - Aging is associated with impaired stress granule formation in kidney cells
Ossama Moujaber

40 - AKT Mediates Ang-II-Induced Phosphorylation and Nuclear Export of Histone Deacetylase 5 in Vascular Smooth Muscle Cells
Vanessa Truong

41 - Defining the cardiac DPP4 cleavage peptidome
Natasha Trzaskalski

42 - Hedgehog Interacting Protein Overexpression in Renal Proximal Tubules Accelerates Renal Dysfunction in Mice Fed with High Fat Diet
Henry Nchienzia

43 - High Fat Diet Leads to Alterations of Hepatic Lipid and Amyloid β Metabolism in the 3xTg-AD Mouse Model of Alzheimer’s Disease
Cristina R. Bosoi

44 - Lack of Tubular Heterogeneous Nuclear Ribonucleoprotein F (hnRNP F) Attenuates Kidney Hypertrophy and Glomerular Hyperfiltration in Diabetic Akita mice
Kana N. Miyata

45 - Littératie alimentaire et le diabète de type 1
Alexandra Itzkovitz

46 - NRF2 Overexpression In The Renal Proximal Tubules Up-Regulates BMF Expression and Promotes Bmf Induced Apoptosis.
Anindya Ghosh

47 - The development of an animal diet more representative of human consumption reveals a major role of dietary proteins in the development of obesity and type 2 diabetes
Béatrice. S.-Y.Choi

48 - The role of PGC-1α in NAFLD-associated liver-cancer.
Mélissa Léveillé
1 - Alginate Beads with Superparamagnetic Iron Oxide Nanoparticles for Pancreatic Islets Encapsulation and Imaging.

John Jahchan\textsuperscript{1}, Philippe Legros\textsuperscript{2}, Marc-André Fortin\textsuperscript{3}, Corinne Hoesli\textsuperscript{4}

\textsuperscript{1}McGill University, \textsuperscript{2}Université Laval, \textsuperscript{3}Université Laval, \textsuperscript{4}McGill

Today, the long-term clinical outcome of islet transplantation through the Edmonton Protocol is similar to that of a full pancreas transplant: 50-70\% of patients achieve insulin independence for 5 years. However, this promising therapy is limited by the immunosuppression required to avoid graft rejection. By encapsulating the islets in alginate beads and administering them to immunocompetent diabetic rodents, diabetes was reversed without the need for immune suppression. Alginate is a porous hydrogel that can block the immune cells and antibodies from going inside the bead but allow the secreted insulin to exit and the oxygen and nutrients to enter by diffusion. Due to the increased graft volume created by the microbeads, microencapsulated islets are typically implanted into the peritoneal cavity. One limitation of microencapsulated islet grafts is that these microbeads may agglomerate or be located far from blood vessels after implantation, leading to islet losses due to hypoxia. The objective of this project is to develop a method to track alginate microbeads using magnetic resonance imaging (MRI) in order to determine the distribution of encapsulated islets in vivo.

This project investigates the addition of Superparamagnetic Iron Oxide Nanoparticles (SPION) developed by our collaborators at the Université Laval to the alginate beads. These nanoparticles are ultra-small which allows positive contrast in MRI in contrary to the classic negative contrast iron oxide nanoparticles. They are synthesized with a novel thermal decomposition protocol. Using an emulsification and internal gelation encapsulation process the beads and nanoparticles can be co-encapsulation with islets. The SPIONs are MRI contrast agents that will enable to track and monitor the graft performance by non-invasive image. The SPION were incubated with MIN6 cells (a mice beta cell line) for 15 hours at different concentrations. The cell cytotoxicity was tested by flow cytometry with propidium iodide stain. The cell viability was found to be more than 90\% for all the three concentrations of SPION (0.5mM, 1mM, 2mM). The nanoparticles were encapsulated in alginate beads and demonstrated strong positive contrast images in different settings: 96 well plates and Eppendorf tubes.

The next objective is to make more robust cytotoxicity and cell function assessments as well as move to a pseudo-islet model developed previously to assess the effect of the nanoparticles on the function and survival of the islets.
2 - Caractérisation in vivo d’un nouveau lien unissant le stress oncogénique à la régulation du cycle cellulaire.

Audrey Poirier1,2, romain villot1,2, Yves Gélinas1,2, Philippe Joubert1,2, Mathieu Laplante1,2

1CRIUOCPQ, 2Université Laval

Le cancer est la principale cause de décès au Canada. La protéine Ras est une petite GTPase fréquemment mutée dans les cancers induisant une prolifération non-contrôlée. Le stress occasionné induit de la sénescence via le suppresseur de tumeur p53. Cette réponse, nommée sénescence induite par les oncogène (SIO) protège les cellules à risque contre l’initiation et la progression tumorale. La protéine BRCA1 joue un rôle important dans la SIO induite par Ras, mais les liens directs entre BRCA1 et Ras ne sont pas encore parfaitement élucidés. Récemment, nous avons identifié ZNF768 comme une nouvelle cible négative de la voie oncogénique Ras. La perte de ZNF768 induit l’arrêt du cycle cellulaire et de la sénescence chez les cellules. Parallèlement, la surexpression de ZNF768 permet de bloquer significativement la SIO induite par Ras de façon p53 dépendante. De plus, ZNF768 interagit avec plusieurs protéines connues pour jouer un rôle dans la SIO de Ras dont BRCA1. Basé sur ces données, nous proposons un modèle dans lequel la déplétion de ZNF768 suite à l’activation de Ras permet d’induire la SIO et prévenir la formation de tumeur via BRCA1.

L’objectif de ce projet consiste à définir les fonctions de ZNF768 in vivo et de confirmer son implication dans la régulation du cycle cellulaire et la tumorigénèse. ZNF768 fut inactivé chez la souris par la technique CRISPR. Le développement, la radiosensibilité ainsi que la susceptibilité aux tumeurs ont été étudiés dans ce modèle. De plus, des mouse embryonic fibroblasts (MEFs) ont été isolés à partir d’embryons et le processus d’immortalisation caractérisé. Les souris ZNF768 KO sont plus petites que les souris contrôles ce qui indique un défaut de croissance. Les MEFs KO entrent en sénescence réplicative plus rapidement lors du processus d’immortalisation. Ces phénomènes concordent avec notre hypothèse et sont observés dans d’autres modèles de souris KO de régulateurs positif du cycle cellulaire. La perte de ZNF768 n’affecte pas la radiosensibilité, ni la susceptibilité aux tumeurs des souris. Nous avons toutefois observé que les niveaux de ZNF768 sont augmentés dans les tumeurs chez la souris et chez l’humain. Il est donc possible que la surexpression de ZNF768 dans les tumeurs soit un mécanisme acquis pour bloquer la SIO. Nous travaillons présentement au développement d’un modèle de souris surexprimant ZNF768 pour la suite de nos études.
3 - Regulation of glucocorticoid receptor turnover by USP19 deubiquitinating enzyme

Erin Coyne¹, Nathalie Bédard², Simon Wing³

¹McGill University, ²McGill University, ³McGill University

Glucocorticoid signaling is an important signaling node in metabolism. We have previously shown that the inactivation of the deubiquitinating enzyme USP19 results in decreased muscle atrophy, liver gluconeogenesis, and adipogenesis; processes that all involve glucocorticoid signaling. Here, we show that inactivation of USP19 results in a 50% decrease in glucocorticoid receptor (GR) protein levels in skeletal muscle, liver, and adipose tissue compared to wild-type mice. This decrease in GR protein levels is not associated with a decrease in GR mRNA levels. Overexpression of the ER-localized or cytosol-localized isoforms of USP19 in cells results in increased levels of GR protein, while knock-out of USP19 by CRISPR-Cas9 results in decreased levels of GR protein. In addition, GR is more ubiquitinated and more rapidly degraded in USP19 KO cells. Importantly, USP19 and GR interact as shown by immunoprecipitation and proximity ligation assays. Interestingly, the N-terminal CS domains of USP19 are important for the regulation of GR protein levels, while the catalytic activity of the enzyme is not. Together, these studies suggest that GR is a novel target of USP19 and that developing pharmacological inhibitors of USP19 could be a beneficial approach to treating conditions such as muscle wasting or obesity.
4 - ZNF768 connecte la signalisation des facteurs de croissance au contrôle de la prolifération.

Villot R\textsuperscript{1}, Poirier A\textsuperscript{1}, Bakan I\textsuperscript{1}, Bérubé JC\textsuperscript{1}, Coulombe Y\textsuperscript{1}, Gélinas Y\textsuperscript{1}, Caron D\textsuperscript{2}, Gobeil S\textsuperscript{2}, Elowe S\textsuperscript{2}, Bossé Y\textsuperscript{1}, Masson J.Y\textsuperscript{2}, Bilodeau S\textsuperscript{2}, Laplante M\textsuperscript{1}

\textsuperscript{1}Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec (CRIUCPQ), Faculty of Medicine, Laval University, 2725 Chemin Ste-Foy, Québec, Qc, Canada, G1V 4G5, \textsuperscript{2}Laval University Medical Research Center, CHUQ, Faculty of Medicine, Laval University, 2705 Boulevard Laurier, Québec, Qc, Canada, G1V 4G2

RAS est une petite protéine Rho-GTPase au sommet de la signalisation des facteurs de croissance qui stimule de nombreuses voies prolifératives et anaboliques telles que les MAPs kinases et l’axe PI3-Kinase/AKT. Des mutations oncogéniques dans la voie RAS comme dans la PI3-Kinase sont fréquentes dans le cancer et associées à une forte agressivité des tumeurs ainsi qu’à une baisse d’efficacité de la chimiothérapie. En nous basant sur des données de phosphoprotéomique, notre équipe a identifié ZNF768, une nouvelle protéine à doigts de zinc jamais caractérisée. Nous avons démontré que l’ensemble de l’axe RAS/MAPK/PI3-Kinase/AKT converge vers une régulation négative de ZNF768, au niveau post-traductionnel. La déplétion aiguë de ZNF768 entraîne une chute massive de l’index mitotique, suivie d’une entrée en sénescence ou d’une mort cellulaire par apoptose. Récemment, des expériences de BioID (Proximity-dependant biotin identification) ont été réalisées dans le but d’identifier des partenaires protéiques potentiels de ZNF768. Les données produites indiquent une association claire entre celle-ci et la signalisation impliquant la réponse aux dommages à l’ADN et le contrôle du cycle cellulaire. De manière intéressante, nous avons constaté que les niveaux de ZNF768 sont rapidement réduits suite à l’induction de dommages à l’ADN d’une façon dépendante des voies RAS/MAPK et PI3K/AKT. Enfin, de récentes analyses indiquent que la surexpression de ZNF768 réduit la sénescence induite par les oncogènes d’une manière dépendante à l’activité du suppresseur de tumeur TP53. Le contrôle du cycle cellulaire par l’axe RAS/MAPK/PI3-Kinase/AKT et son implication dans l’entrée en sénescence est bien établie mais reste peu compris. Ainsi, l’ensemble de ces données suggère que cette nouvelle protéine à doigts de zinc pourrait être un nouveau lien entre la signalisation des facteurs de croissance et la prolifération cellulaire.
5 - 14-3-3ζeta is required for PKA-dependent lipolysis

Abel Oppong¹, Yves Mugabo¹, Gareth Lim¹
¹CRCHUM/ Université de Montréal

The molecular scaffold, 14-3-3ζ, was previously found to be essential for visceral adipogenesis, but its contributions to the function of mature adipocytes is not known. As it can regulate the activities of metabolic effectors, we hypothesized that 14-3-3ζ also has essential roles in adipocyte function.

3T3-L1 adipocytes and mouse models were used to study if 14-3-3ζ regulates lipolysis. Depletion of 14-3-3ζ by siRNA abrogated glycerol and free fatty acid (FFA) release from 3T3-L1 cells treated with Isoproterenol (ISO, 1 μM), Forskolin (FSK, 10 μM), and dibutyryl cAMP (1 mM). In contrast, over-expression of 14-3-3ζ potentiated ISO-mediated FFA release. Knockdown of 14-3-3ζ did not affect cAMP generation in ISO- and FSK-treated 3T3-L1 cells, but mRNA levels of lipases (Atgl, Hsl, and Magl) and Pparg were reduced, suggesting a loss of adipocyte identity. Decreased activation and total expression of PKA substrates, including Hsl and CREB, were detected in 14-3-3ζ-depleted 3T3-L1 cells. Taken together, these data suggest that 14-3-3ζ is necessary for lipolysis from 3T3-L1 adipocytes.

To understand adipocyte-specific roles of 14-3-3ζ, tamoxifen (TMX)-inducible, adipocyte-specific 14-3-3z knockout (adi14-3-3zKO) mice were used. Four weeks after TMX exposure (5 days, 50 mg/kg), no effects on body weight were found. After an overnight fast, adi14-3-3zKO mice displayed impaired lipolysis following i.p CL-316,243 (1 mg/kg) injections. In contrast, transgenic over-expression of 14-3-3ζ did not affect lipolysis. Adi14-3-3zKO mice also displayed glucose intolerance following i.p. glucose (2 g/kg). Real-time PCR confirmed significant reductions in Atgl, Hsl, and Pparg mRNA levels in adi14-3-3zKO mice, suggesting impaired adipocyte function.

Collectively, these results demonstrate essential functions of 14-3-3ζ in facilitating lipolysis and, potentially, adipocyte maturity. Future studies are aimed at understanding how 14-3-3ζ regulates other aspects of adipocyte function, including diet-induced expansion of fat mass.
6 - Adipose α/β-hydrolase domain-6 is a negative modulator of adipose thermogenesis and its inhibition promotes metabolically healthy obesity

Pegah Poursharifi\textsuperscript{1}, Camille Attané\textsuperscript{1}, Yves Mugabo\textsuperscript{1}, Anfal Almass\textsuperscript{1,2}, Shangang Zhao\textsuperscript{1}, Roxane Lussier\textsuperscript{1}, Heidi Erb\textsuperscript{1}, Julian Guida\textsuperscript{1}, Elite Possik\textsuperscript{1}, Marie-Line Peyot\textsuperscript{1}, Erik Joly\textsuperscript{1}, Andre Tchernof\textsuperscript{3}, Christophe Noll\textsuperscript{4}, Andre C. Carpentier\textsuperscript{4}, S.R. Murthy Madiraju\textsuperscript{1}, Marc Prentki\textsuperscript{1}

\textsuperscript{1}CRCHUM, \textsuperscript{2}McGill University, \textsuperscript{3}Université Laval, \textsuperscript{4}Université de Sherbrooke

Obesity is a rapidly growing threat to the global health and activation of energy expenditure processes in the brown adipose tissue (BAT) and white adipose tissue (WAT) may provide solutions. Pan-deletion of the lipase alpha/beta-domain hydrolase-6 (ABHD6) demonstrated the therapeutic potential of ABHD6 inhibitors against obesity and type-2-diabetes; though the precise depot-specific role of ABHD6 in the adipose tissue metabolism remains unexplored.

ABHD6\textsuperscript{floxflo} mice were bred with Adipoq-Cre/ERT2 mice and Abhd6\textsuperscript{floxflo}/Adipoq-Cre to obtain AT-ABHD6-KO mice by tamoxifen injection. Control littermates and KO mice were fed a normal diet (ND) or a high fat diet (HFD) for 12 weeks. For thermogenesis studies, ND mice were kept either at room temperature or exposed to cold temperature (4°C and 10°C) for 24h - 48h.

Our results show that adipose tissue ABHD6 expression level increases during adipocyte differentiation, and correlates with adiposity in WAT from HFD mice and in visceral fat depot from patients with obesity. AT-ABHD6-KO mice on ND showed similar phenotype as controls at room temperature, but were resistant to cold induced hypothermia and displayed reduced adipocyte size and inflammation. The enhanced energy expenditure in cold was in part due to accelerated glycerolipid/fatty acid futile cycle in visceral adipose and oxidative metabolism in BAT. Under cold-exposure 2-monoacylglycerol (MAG) levels were increased in the WAT from KO mice. The mRNA expression of PPAR alpha/PPAR gamma target genes were induced by 2-MAG treatment. In addition, we found that 2-MAG is also capable of activating PPAR alpha in transactivation studies, suggesting that PPAR activation by accumulating 2-MAG contributed to thermogenic mechanisms in the cold exposed AT-ABHD6-KO mice. KO mice on HFD displayed healthy-obese characteristics, including improved insulin sensitivity, elevated WAT beta-oxidation, lower liver TG content, and improved systemic and WAT inflammation.

The results indicate that ABHD6 negatively modulates the adipose thermogenic program and AT-ABHD6 deletion protects from cold induced hypothermia and also promotes a healthy-obese phenotype in HFD fed mice.
7 - Development of a method to identify new regulatory processes controlling GLUT4 translocation

Nolwenn Samson¹, André Marette¹, Mathieu Laplante¹

¹Université Laval

Introduction: The insulin signalling pathway is involved in numerous biological processes, such as cell growth, lipid metabolism and glucose homeostasis. Impaired insulin signalling is present in metabolic disorders like obesity and type 2 diabetes, which leads to hyperglycemia and insulin resistance. GLUT4 is a glucose transporter encoded by the Solute carrier family 2 member 4 (SLC2A4) gene, and is mainly expressed in skeletal muscle and adipose tissue. At the basal state, GLUT4 is contained in specific compartments named GSVs (GLUT4 storage vesicles). By binding to its receptor, insulin triggers GLUT4 translocation to the plasma membrane and thereby the absorption of glucose in myocytes and adipocytes. Several studies have shown that this transporter is essential to maintain glucose homeostasis and that increasing its expression in diabetic animals improves glucose metabolism. We hypothesize that there are still unknown factors involved in GLUT4 translocation that could be targeted to promote glucose uptake in a context of insulin resistance.

Methods: To create an insulin-resistant environment, we used the CRISPR-Cas9 technology to induce a knock-out (KO) of the insulin receptor (IR) in C2C12 myoblasts. A guide RNA targeting the exon 2 of the IR gene was inserted in a plasmid containing a spCas9 and a puromycin resistance. The construct has been nucleofected in C2C12 cells and the cells selected with puromycin. Since the C2C12 cell line has a near tetraploid karyotype, we performed serial dilutions of our initial population in order to obtain one cell per well in 96-well plates, and then isolate clones with a complete IR-KO. PCR, sequencing and Western Blot were used to confirm the KO. Cells were next infected with a reporter vector (pLenti-myc-GLUT4-mCherry) in order to visually study GLUT4 translocation. This vector allows the quantification of GLUT translocation using FACS.

Results: Several IR-KO clones were obtained following Cas9 nucleofection in C2C12 myoblasts. Insulin dose-response realized in one IR-KO clone showed an inactivation of the IRS-1/PI3K/Akt pathway, as expected. First pictures of C2C12 infected with pLenti-myc-GLUT4-mCherry by fluorescence microscopy showed an equal intracellular GLUT4 distribution. Over the next months, flow cytometry and cell sorting will be used to isolate insulin resistant clones with high membrane GLUT4 levels. Gene expression profile comparing various clones may reveal new pathways regulating GLUT4 translocation independently of the IR.

Conclusion: Using the tools that we have developed, we hope to discover new targets regulating GLUT4 translocation. Our work could allow the development of new therapeutic avenues to improve glucose metabolism in insulin resistant and diabetic patients.
Effet de l’absence de la protéine IGFBP-2 sur le profile lipidique et le développement de la plaque d’athérome chez des souris femelles.

Chloé Rauzier1,2, Stéphanie Miard1, Jean-Pierre Després1,3, Frédéric Picard1,2

1Centre de Recherche de l’Institut Universitaire de Cardiologie et Pneumologie de Québec, 2Faculté de Pharmacie, Université Laval, 3Faculté de Médecine, Université Laval

PROBLÉMATIQUE : La protéine circulante IGFBP-2 est une protéine principalement produite par le foie et impliquée dans de nombreux processus biologiques en lien avec le vieillissement et les maladies cardiométaboliques. Une récente étude a montré que les niveaux circulants d’apoB-LDL, lipoprotéine hautement athérogène, sont négativement associés de manière forte et indépendante aux niveaux plasmatiques d’IGFBP-2. Des niveaux faibles d’IGFBP-2 pourraient ainsi favoriser le développement de la plaque d’athérome. OBJECTIFS : Évaluer si l’absence de la protéine IGFBP-2 chez la souris femelle module le développement et la progression de la plaque d’athérome. MÉTHODOLOGIE : Des souris femelles Igfbp-2+/+ et Igfbp-2-/- sur fond génétique C57BL/6J ont été nourries durant 24 semaines avec une diète pauvre ou riche en gras. RÉSULTATS : La variation du poids des souris dans le temps n’a pas été différente entre les génotypes ; en revanche l’effet de la diète a été bien marqué. Les souris Igfbp-2-/- ont eu une glycémie plus haute que celle des souris Igfbp-2+/+ après injection d’insuline. En dépit de ce résultat, aucune différence significative n’a été constatée entre les génotypes pour ce qui est des niveaux plasmatiques de cholestérol, de triglycérides, d’acides gras non-estérifiés et de glucose. En revanche une diminution des niveaux hépatiques de cholestérol a été observée chez les souris Igfbp-2-/-.


Étude financée par la Fondation de l’IUCPQ.
9 - Identification d'une nouvelle cible de PCSK9: Dégradation du récepteur de l'insuline dans le foie de modèles murins par PCSK9

Julie Cruanes1,4, J. L. Estall2,3,4, N. G. Seidah1,3,4

1Unité de Biochimie Neuroendocrinienne, Institut de Recherches Cliniques de Montréal, 2Unité sur les mécanismes moléculaires du diabète, Institut de Recherches Cliniques de Montréal, 3Faculté de Médecine, Université de Montréal, 4Faculté de Médecine, Université de McGill

La régulation du récepteur des lipoprotéines de basse densité (LDLR) par la proprotéine convertase subtilase kéxine de type 9 (PCSK9), une protéine circulante de la famille des proprotéines convertases (PC), est centrale à l'homéostasie du cholestérol. L'effet de la diminution de PCSK9 dans le plasma est controversé car il est probable que celle-ci mène à une augmentation de l'internalisation de facteurs toxiques dans les tissus périphériques, tels que les tissus pancréatiques endocriniens, et donc le développement du diabète de type 2. En outre, il y a plusieurs études épidémiologiques qui rapportent une corrélation positive entre les niveaux circulants de PCSK9 et le risque pour le diabète. Des résultats préliminaires de notre laboratoire indiquent une interaction entre PCSK9 et le récepteur de l’insuline (IR) in vitro. Nous proposons donc que le récepteur de l’insuline (IR) serait une cible de PCSK9. Des niveaux élevés de PCSK9 diminueraient ainsi la signalisation de l’insuline.

Objectifs : Vérifier in vitro et in vivo que PCSK9 diminue les niveaux totaux du IR et la signalisation de l’insuline.

Méthodes : In vitro, l’incubation d’un milieu concentré en PCSK9, en présence ou absence d’insuline, sur des hépatocytes humaines immortalisées permet de faire des analyses de colocalisation du IR avec PCSK9 (microscopie confocale), de régulation de la voie de signalisation de l’insuline (immunobuvardage de type Western), ainsi que la quantification du IR à la membrane plasmique (cytométrie en flux). In vivo, l’injection de PCSK9 puis de l’insuline dans des souris mâles de 4 mois permet de valider les résultats in vitro en suivant les mêmes méthodes d’analyses.

Résultats: PCSK9 diminue les niveaux endogènes du IR ainsi que sa voie de signalisation.

Conclusions: Principalement connu pour réguler le cholestérol, PCSK9 diminue aussi les niveaux endogènes du IR ainsi que sa voie de signalisation, possiblement en intervenant dans le transport du IR le redirigeant vers les lysosomes et empêchant son recyclage à la membrane plasmique.
10 - Modulation de la production hépatique d'IGFBP-2 par FGF19.

Justine FARAMIA\textsuperscript{1,2}, Stéphanie MIARD\textsuperscript{2}, Frédéric Picard\textsuperscript{1,2}

\textsuperscript{1}Université Laval, \textsuperscript{2}Centre de Recherche de l'Institut Universitaire de Cardiologie et Pneumologie de Québec

INTRODUCTION : La protéine de liaison aux facteurs de croissance analogues à l'insuline 2 (IGFBP-2) est une protéine circulante majoritairement sécrétée par le foie. Il a été démontré qu'IGFBP-2 est retrouvée en faible concentration en cas de désordres métaboliques (obésité, stéatose hépatique non alcoolique et diabète) et que la chirurgie bariatrique induit une augmentation forte et soutenue de son expression hépatique et de sa concentration plasmatique. Or, les mécanismes de cette augmentation sont à ce jour méconnus. L'une des pistes est la modulation d'IGFBP-2 par le facteur 19 de croissance des fibroblastes (FGF19), dont les concentrations sont aussi stimulées par la chirurgie bariatrique et qui pourrait agir au foie via son récepteur FGFR4.

OBJECTIF : L'objectif de l'étude était donc de déterminer si FGF19 module positivement IGFBP-2 dans des hépatocytes en culture.

MÉTHODOLOGIE : Des hépatocytes humains HepG2 ont été traités, en présence ou en absence de sérum, avec du FGF19 recombinant humain et un inhibiteur spécifique de son récepteur FGFR4, selon des courbes croissantes de doses et temps. L'expression du gène Igfbp2 a été mesurée par qPCR, tandis que la concentration protéique intra et extracellulaire d'IGFBP-2 a été déterminée par immunobuvardage.

RÉSULTATS : Le traitement au FGF19 n'a pas modulé la production hépatique d'IGFBP-2 en présence ou en absence de sérum. Cependant, en privation de sérum, le blocage de la voie FGFR4 a entraîné l'augmentation de l'expression d'IGFBP-2 (ARNm) mais a aussi diminué sa quantité intra et extracellulaire. En présence de sérum, l'inhibiteur du récepteur FGFR4 a diminué significativement l'expression et la production d'IGFBP-2.

CONCLUSION : Nos résultats démontrent que FGF19 ne module pas directement la production hépatique d'IGFBP-2. Cependant, la voie FGFR4 est impliquée dans la régulation d'IGFBP-2.

Étude financée par les IRSC.
**11 - Normocholesterolemic subjects with lower plasma PCSK9 display white adipose tissue dysfunction and activated IL-1β system**

Yannick Cyr¹, Simon Bissonnette¹, Valérie Lamantia¹, Viviane Provost¹, Marie Devaux¹, Gaetan Mayer², Michel Chrétien³, May Faraj¹

¹Université de Montréal/IRCMA, ²Université de Montréal/Institut de cardi, ³University of Ottawa/OHRC

Objective: Plasma LDL-cholesterol lowering genetic variants, such as those causing a PCSK9 loss-of-function, are associated with an elevation in type 2 diabetes (T2D) risk. In fact, PCSK9 causes the degradation of the LDLR, CD36, and other apoB-lipoprotein receptors on tissue surface. These observations suggest that internalization of apoB-lipoproteins through these receptors could play a role in the pathophysiology of T2D. Our team has demonstrated that the NLRP3 inflammasome, in addition to being implicated in white adipose tissue (WAT) dysfunction, is activated by native LDL (NLRP3: NOD-like Receptor Pyrin containing domain 3). We propose that normocholesterolemic subjects with lower circulating PCSK9 have higher CD36 and LDLR expression with higher NLRP3 inflammasome activity in WAT, and more risk factors for T2D.

Method: A cohort of 16 post-menopausal women and 13 men (BMI>25kg/m², LDLC≤3.5mM) was separated in groups based on median plasma PCSK9 by sex. A WAT biopsy was collected at fasting and 4h following ingestion of a high fat meal. Surface expression of LDLR and CD36 was measured for each biopsy in addition to NLRP3 and pro-IL-1β expression and IL-1β secretion. WAT function was measured ex vivo as the hydrolysis and storage of a synthetic ³H-triolein-labeled triglyceride-rich lipoprotein substrate.

Result: Compared to subjects with higher plasma PCSK9 (296±17 ng/ml), subjects with lower plasma PCSK9 (201±11 ng/ml) had higher WAT surface LDLR (+78%) and CD36 (+41%) expression. They also displayed lower expression of pro-IL-1β (-44%) at fasting but greater 4h-postprandial increase (+46%), as well as increased fasting WAT IL-1β secretion (+179%). Finally, these subjects also had decreased WAT function (-60%) and higher plasma IL-1Ra (+74%).

Conclusion: We propose that receptor-mediated apoB-lipoprotein internalization in WAT favors development of dysfunction, activation of the NLRP3 inflammasome, and increased risk of T2D.
12 - Neurobehavioral correlates of obesity are largely heritable

Uku Vainik1,2, Travis E. Baker3, Mahsa Dadar1, Yashar Zeighami1, Andréanne Michaud1, Yu Zhang1, José C. García Alanis4, Bratislav Misić1, D. Louis Collins1, Alain Dagher1

1Montreal Neurological Institute, McGill University, Montreal, QC H3A 2B4, Canada; 2Institute of Psychology, University of Tartu, Näituse 2, 50409 Tartu, Estonia, 3Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102, 4Neuropsychology Section, Experimental and Biological Psychology, Department of Psychology, Philipps University of Marburg, 35032 Marburg, Germany

Recent molecular genetic studies have shown that the majority of genes associated with obesity are expressed in the central nervous system. Obesity has also been associated with neurobehavioral factors such as brain morphology, cognitive performance, and personality. Here, we tested whether these neurobehavioral factors were associated with the heritable variance in obesity measured by body mass index (BMI) in the Human Connectome Project (n = 895 siblings). Phenotypically, cortical thickness findings supported the “right brain hypothesis” for obesity. Namely, increased BMI is associated with decreased cortical thickness in right frontal lobe and increased thickness in the left frontal lobe, notably in lateral prefrontal cortex. In addition, lower thickness and volume in entorhinal-parahippocampal structures and increased thickness in parietal-occipital structures in participants with higher BMI supported the role of visuospatial function in obesity. Brain morphometry results were supported by cognitive tests, which outlined a negative association between BMI and visuospatial function, verbal episodic memory, impulsivity, and cognitive flexibility. Personality–BMI correlations were inconsistent. We then aggregated the effects for each neurobehavioral factor for a behavioral genetics analysis and estimated each factor’s genetic overlap with BMI. Cognitive test scores and brain morphometry had 0.25–0.45 genetic correlations with BMI, and the phenotypic correlations with BMI were 77–89% explained by genetic factors. Neurobehavioral factors also had some genetic overlap with each other. In summary, obesity as measured by BMI has considerable genetic overlap with brain and cognitive measures. This supports the theory that obesity is inherited via brain function and may inform intervention strategies.
13 - Modulation of the insulin-like growth factor-1 axis after bariatric surgery in morbidly obese patients

Meng Li¹, Audrey Auclair¹, Chloe Anais Rauzier¹, Frederic Picard¹
¹Université Laval

Background and aim: Bariatric surgery is the most effective long-term therapy for metabolic changes in patients with severe obesity. Exercise can help to preserve muscle mass, increase muscle protein synthesis and prompt insulin sensitivity following the remarkable weight loss induced by a bariatric procedure. Several studies reported an association between serum IGF1 level and its binding proteins (IGFBPs) in obese patients. To date, there has not been a population-based study demonstrating the impact of different types of bariatric surgery and exercise on the total and free IGF-1 (fIGF-1), and their relationship to IGFBP-1, IGFBP-2, IGFBP-3, insulin in severe obesity whose BMI ≥ 40 kg/m² or BMI ≥ 35 kg/m² with co-morbid conditions. The aim of the study was to address these issues in a retrospective cohort of morbidly obese patients.

Methods: The patients (n=59) were divided into Biliopancreatic diversion with duodenal switch group (n=28) and Sleeve Gastrectomy group (n=31) at baseline. And then were randomized into two groups, with supervised exercise group (n=40) and without exercise group (n=19) at the third month following bariatric surgery. Those in the exercise group underwent a 12-week aerobic exercise physical training program at the 3 months following the surgery. The Medical history, anthropometric measurements and tissue volume of the abdomen and thigh were performed before, at 3 months, 6 months and 1 year after the surgery. The blood samples were taken to measure lipids, glucose, insulin and IGF-axis proteins at each time point.

Results: We first documented the clinical characteristic of patients before and after the bariatric surgery among the four groups. A total of 59 patients were included, the mean ± s.e.m age of the patients in this cohort at baseline was 41.8 ± 1.5 years. Mean ± s.e.m BMI was 46.1 ± 0.8 kg/m2, fat mass was 62.4 ± 1.7 Kg and fat-free mass was 63.7 ± 1.6 Kg. The concentrations of the IGF-1 and insulin across the four time-points are independent of the surgery*exercise interaction. But the concentration of fIGF-1 across the four points are dependent upon the types of bariatric surgery (fig. 1). IGF-1 reduced less in SG groups than BPD-DS groups at 3-month. And the IGF-1 levels decrease in exercise groups after 12 months following both types of bariatric surgeries compared to preoperative levels. Meanwhile, the IGF-1 levels were greater at 12-month compared with baseline in no exercise groups (fig. 1A). From the SG with exercise group and BPD-DS with exercise group, the type of surgery expand difference of fIGF-1 levels. And irrespective of the presence or absence of the exercise, SG groups have higher concentrations of fIGF-1 than BPD-DS groups (fig. 1C). Meanwhile, IGFBP-1 and -2 shows the converse results compared to fIGF-1. The reduction of insulin levels following BPD-DS groups are more than SG groups at any time point (fig. 1E). The figure 2 shows concentrations of the IGFBP-3 across the four time points are independent on the surgery * exercise interaction. And the area under the curve of IGFBP-3 suggests that there is no significant difference among the four groups (fig. 2F). But the concentration of IGFBP-1 and IGFBP-2 across the four points are dependent upon the types of bariatric surgery. Furthermore, BPD-DS with exercise group increase greater than BPD-DS without exercise group at 6-month and 12-month.

Conclusion: Plasma levels of IGFBP-2 increase following the different types of surgery and this modulation is greater compared to IGFBP-1. And irrespective of the presence or absence of the exercise, BPD-DS have higher concentrations of IGFBP-1 and -2 than SG. And fIGF-1 shows the converse results following the bariatric surgery. Moreover, physical activity has additive beneficial effect on IGFBP-2 after bariatric surgery.
14 - Omega-3 fatty acids inhibit the interleukin-1β pathway in white adipose tissue and correlate with an improvement in C-peptide secretion in humans

Valérie Lamantia¹,², Simon Bissonnette¹,², Yannick Cyr¹,², Viviane Provost³, Marie Devaux², May Faraj¹,²

¹Université de Montréal, ²Institut de recherches cliniques de Montréal, ³Centre hospitalier de l'Université de Montréal

Objective: Activation of the interleukin-1β (IL-1β) pathway induced by the NLRP3 inflammasome promotes white adipose tissue dysfunction (WAT) and type 2 diabetes (T2D) in humans. Omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), ameliorate insulin resistance in humans and inhibit IL-1β secretion in murine WAT, but their effect on the IL-1β pathway in human remains unclear. We tested the hypothesis that EPA and DHA inhibit the IL-1β pathway in human WAT in vivo and ex vivo.

Methods: We examined 33 non-diabetic subjects (45-74 years, >20 kg/m², 64% postmenopausal women) before and after 12-week supplementation of 1.6 g EPA and 1.1 g DHA/day. Glucose-induced insulin and C-peptide secretions and insulin sensitivity were measured during a 3h-Botnia clamp. Fasting red blood cell (RBC) phospholipid EPA and DHA were measured by gas chromatography mass spectrometry. Protein expression of NLRP3, pro-IL-1B and procaspase-1 were measured in fasting WAT by immunoblot. The IL-1β secretion from fasting WAT was measured by alpha-LISA. Direct effect of EPA+DHA (200 mmol/L) on fasting WAT IL-1β secretion was examined over 7 hours following WAT stimulation with lipopolysaccharide (LPS) and adenosine triphosphate (ATP) (positive controls for maximum secretion) or native LDL (metabolic signals).

Results: At baseline, %EPA+DHA in RBC phospholipids was associated negatively with WAT IL-1β secretion (r=-0.46, p=0.029). There was a significant increase in %EPA+DHA in RBC phospholipids (baseline=1.62±0.74% vs post-intervention=3.75±1.93%, p<0.001). Best responders to EPA:DHA to inhibit WAT NLRP3 inflammasome activity were subjects with higher baseline activity, as post-intervention changes in pro-IL-1β, procaspase-1 and IL-1β secretion were inversely correlated to their baseline levels (r=-0.64, r=-0.63 and r=-0.72 respectively, p<0.01). Moreover, post-intervention change in WAT IL-1β secretion correlated positively with that of C-peptide secretion (r=0.58, p=0.048). Addition of EPA+DHA inhibited IL-1β secretion by LPS+ATP-stimulated (-88.6%, p <0.05) or LDL-stimulated WAT (-81.4%, p<0.01).

Conclusion: The improvement in C-peptide secretion following EPA and DHA supplementation is associated with the amelioration in WAT IL-1β pathway. Omega-3 supplementation may aid in the prevention of T2D in subjects with activated WAT IL-1β pathway.
15 - Risk of cardiometabolic complications in cystic fibrosis patients who are overweight or obese and those who go through a significant weight change

Anne Bonhoure¹,3, Valerie Boudreau¹,2, Johann Colomba¹,2, Cindy Bergeron¹,4, Marjolaine Maillot⁵, François Tremblay¹,5, Annick Lavoie¹,5, Rémi Rabasa-Lhoret¹,2,3,5

¹Institut de recherches cliniques de Montréal, Montréal, Canada, ²University of Montreal, Faculty of Medicine, Department of Nutrition, Montréal, Canada, ³McGill University, Department of Medicine, Experimental Medicine Division, Montréal, ⁴University of Montreal, Faculty of Medicine, Department of Pharmacology/Physiology, Montréal, ⁵Cystic Fibrosis Clinic, Centre Hospitalier Universitaire de Montréal (CHUM), Montréal, Canada

Objectives
Cystic fibrosis (CF) is associated with malabsorption so maintaining a normal body mass index (BMI) is crucial. Measures to improve nutrient intake and absorption have led to a decrease in underweight patients and an increase in normal and overweight/obese patients. A high BMI is associated with better pulmonary function and survival. However, the metabolic impacts of overweight, obesity and significant weight gain are unknown. The aim is to characterize the clinical and cardiometabolic status of adult CF patients who are overweight/obese and go through important weight change.

Methods
Baseline data from a total of 290 adult CF patients from the Montreal CF cohort as well as follow-up (3.5 years) of 158 adults. Nutritional status (NS) groups based on BMI categories, underweight (UW<18.5 kg/m²), normal (NW 18.5 to 26.9 kg/m²), and overweight/obese (OW≥27 kg/m²), and weight change (WC) over time groups, lost (LW>10% weight lost), stable (SW), and gained (GW >10% weight gain), are compared to pulmonary function (FEV) and cardiometabolic status: glucose tolerance, estimated insulin resistance (IR), blood pressure (BP) and inflammation (CRP).

Results
Compared to the UW and NW patients, the OW group is older (22.7±4.9 vs 25.2±7.8 vs 34.0±8.5 yrs., p<0.001), had a lower prevalence of pancreatic insufficiency (88.6 vs. 80.8 vs. 55%, p=0.009), a higher systolic BP (p<0.004) and a higher IR (p<0.001). Compared to UW patients, OW patients also had a higher %FEV₁ (56.2±18.4 vs 82.8±19.6%, p<0.0001) and lower CRP (11.9±11.0 vs 5.3±6.4 mg/L, p=0.007). No differences were observed for glucose tolerance. For weight change, compared to the LW and SW groups, the GW group had a higher IR (p=0.017) and triglycerides (p<0.001) at follow-up. No differences were observed for glucose tolerance.

Conclusions
A higher BMI or weight gain over time is associated with a better pulmonary function but also some unfavorable cardiometabolic parameters.
16 - Role of GAS6 in insulin sensitivity and cholesterol biosynthesis

Céline SCHOTT¹, Amélie Germain², Julie Lacombe², Mathieu Ferron³

¹IRCM-Université de Montréal, ²Institut de recherches cliniques de Montréal, ³IRCM

Vitamin K is the cofactor of γ-carboxylase an enzyme that convert the glutamic acid residues into γ-carboxyglutamic acid residues in some secreted proteins. Clinical data suggest that vitamin K may influence the development of diabetes, but the γ-carboxylated protein(s) and the mechanisms involved remain unknown. Growth arrest-specific 6 (GAS6) is a secreted γ-carboxylated protein that acts as a ligand for the TAM tyrosine kinase receptor family, which includes, TYRO3, AXL, and MERTK. GAS6 and its receptors play a critical role in the immune system, in tumor progression, and in cancer metastasis. More recent studies found that circulating GAS6 levels or SNPs in Gas6 gene are associated with obesity and insulin resistance in humans, however, the mechanism by which GAS6 influences these metabolic disorders is not understood.

We therefore decided to 1) investigate the role of GAS6 in the development of insulin resistance and diabetes in vivo using mouse models; and 2) determine the signalling pathways involved.

We first noticed in wild-type mice that the serum levels of GAS6 are higher in feeding condition compared to fasting, suggesting that the secretion of this factor is regulated by the nutritional and/or the energetic status of the animals. Next we characterize the function of GAS6 in energy metabolism, by analyzing the metabolic phenotype of 3-month-old Gas6⁻/⁻ male mice fed a normal diet. We found that these mice have improved glucose tolerance and insulin sensitivity, but normal insulin secretion, when fed a normal chow diet. This phenotype was also observed at 16 months of age and in young mice fed a high-fat diet, suggesting that the absence of GAS6 protects from age- or diet-induced insulin resistance. Conversely, in a transgenic gain-of-function model (i.e., ApoE-Gas6Tg), increased GAS6 circulating levels is sufficient to cause reduced insulin sensitivity.

TAM receptors gene expression analysis in insulin-sensitive tissues revealed that AXL is highly expressed in skeletal muscle and white adipose tissue. We further show that AXL is activated by GAS6 in myotubes (C2C12) and adipocytes (3T3-L1) in culture. We therefor characterized the GAS6/AXL-dependent transcriptome in C2C12 myotubes following treatment with recombinant GAS6 or a pharmacological inhibitor of TAM receptors (LDC1267). Interestingly, RNA sequencing analysis revealed that GAS6/AXL signalling regulates the expression of genes encoding proteins involved in signalling downstream of the insulin receptor (e.g., Irs2 and Eif4ebp1) and in cholesterol biosynthesis (e.g., Hmcs1, Hmgcr, Nsdhl, Fdft1, etc.). We further show that several of these genes are up-regulated in the skeletal muscle of Gas6⁻/⁻ mice at 16 months of age. In addition, we found a decrease in the cholesterol levels of myotubes treated with GAS6 (200 ng/ml) in vitro. In the presence of a pharmacological inhibitor of AXL (R428), the effect of GAS6 is blunted, suggesting that GAS6 inhibits cholesterol synthesis in an AXL-dependent manner in muscle cells.

Our results suggest that GAS6, via its receptor AXL, may reduce insulin sensitivity by inhibiting the insulin signalling pathway. GAS6 also inhibits cholesterol biosynthesis in muscle cells. Since cholesterol is synthesized from Acetyl-CoA, a product of glycolysis, we propose that GAS6, by decreasing the expression of cholesterol biosynthesis genes, decreases cholesterol formation, inducing a decrease in glucose consumption by the muscle. Altogether, these effect of GAS6 on muscle, could favour hyperglycemia and the development of diabetes.
17 - The Role of 14-3-3ζ in the “beiging” of white adipose tissue.

Kadidia Diallo1,2, Gareth Lim1,2

1CRCHUM, UdeM, 2CRCHUM/ Université de Montréal

Adipocyte hypertrophy and hyperplasia is a hallmark of obesity, and treatments that induce the oxidation of lipid stores may represent a potential therapy. When stimulated by cold or beta-adrenergic agonists, adipocytes in inguinal white adipose tissue (iWAT) can convert to beige cells, which resemble brown adipocytes. This process can reduce body weight in rodents and improve glucose homeostasis through increased Ucp1-dependent lipid oxidation. 14-3-3ζ, a molecular scaffold we found to be essential for adipogenesis, regulates the enzymatic activities of tryptophan and tyrosine hydroxylases, both of which influence beiging. Thus, the aim of the current study is to investigate whether 14-3-3ζ influences the beiging process.

Compared to control mice, acute (3 hr) and chronic (72 hr) cold (4°C) exposure in male transgenic mice over-expressing 14-3-3ζ led to improved cold tolerance due to significantly increased Ucp1 mRNA and protein in iWAT. Following chronic exposure, they were also able to maintain their body weight by increasing their food intake. Consistent with these data, analysis of adipocyte area revealed a decrease in the size of inguinal adipocytes in transgenic mice, suggesting increased lipid oxidation. In contrast, gonadal adipocytes were larger in transgenic mice, which may explain their ability to maintain their body weight. Systemic 14-3-3ζ knockout mice had significantly lower levels of Ucp1 mRNA in iWAT and BAT but did not display differences in tolerance to acute cold. Depletion of 14-3-3z by siRNA in brown adipocytes did not impair isoproterenol-mediated induction of Ucp1 mRNA, suggesting that 14-3-3z is not required for Ucp1 expression in this cell type.

In future studies, we will examine if 14-3-3ζ influences the responsiveness of iWAT to chronic β-adrenergic stimuli and assess the impact of 14-3-3ζ overexpression in brown adipocyte function. Collectively, our results point to a novel role of 14-3-3ζ in the beiging of inguinal adipocytes and increase our understanding of how beiging is regulated.
18 - Vitamin K in adults with cystic fibrosis is correlated to fat mass and insulin secretion

Valérie Boudreau¹², Cindy Bergeron¹², Bouchra Ouliass³, Johann Colomba¹², Anne Bonhoure¹⁴, Marjolaine Mailhot⁵, Annick Lavoie⁵, Guylaine Ferland²³, Rémi Rabasa-Lhoret¹²⁵

¹¹. Institut de recherches cliniques de Montréal, Montréal, Canada, ¹². Département de Nutrition, Faculté de Médecine, Université de Montréal, Montréal, Canada, ³³. Institut de Cardiologie de Montréal, Montréal, Canada, ⁴¹. Division de Médecine Expérimentale, Université McGill, ⁵¹. Clinique de Fibrose Kystique, CHUM, Montréal

Objectives

Cystic fibrosis (CF) is associated with malnutrition, caused by malabsorption (exocrine pancreatic insufficiency (EPI)) and increased energy expenditure. Despite supplementation lipid-soluble vitamin deficiencies are frequent, but the status of vitamin K, which can have a role in insulin secretion, has limited documentations. We have characterized the levels of vitamin K of people afflicted with CF in relation to their clinical and glycemic status.

Methods

Vitamin K₁ was measured using the blood serum of 167 adult patients from the Montréal cystic fibrosis cohort at the time of an oral glucose tolerance test (OGTT: 2 h with plasma glucose and insulin every 30 min). Pulmonary function was also measured at that time using spirometry (FEV1), as well as fat-mass (bio-impedance), body mass index (BMI), and EPI (pancreatic supplement enzymes (PE)). The patients were dichotomized based on vitamin K level: <0.28 nmol/L (lower group) vs. ≥0.29 nmol/L. Data is presented as the mean ± standard deviation with p values (ANOVA or Chi-2).

Results

66% of patients were in the lower vitamin K level group. The proportions between sex and pulmonary function were similar between the 2 groups, but patients from the lower group were younger (24.8±6.7 vs 27.4±9.1 yrs., p=0.041), thinner (BMI: 20.9±2.7 vs 22.7±3.3 kg/m², p<0.001), had a lower fat mass (10.4±5.2 vs 22.7±3.2 %, p<0.001), and took more PE (85 vs 63%, p=000.1). The plasma glucose at the five time points of the OGTT is similar between groups, but the group with lower levels of vitamin K secreted less insulin at all time points (p≤0.05) besides the 90 minute one.

Conclusions

Approximately 2/3 of CF patients have lower levels of vitamin K. These patients are younger, thinner, have less fat mass and secret less insulin. However, the glucose tolerance is not affected.
C– Neurobiology and energy homeostasis / Exercise

19 - Cibler le domaine alpha / bêta-hydrolase 6 dans le contrôle neuronal de l'alimentation et de la récompense

David Lau1,2, Stephanie Fulton1,2

1Université de Montréal, 2CRCHUM

Objectif: Le système endocannabinoïde (eCB) est connu pour réguler l'activité des neurones dopaminergiques de l'aire tegmentale ventrale (VTA). Comme ces neurones sont essentiels pour un comportement motivé, cette étude a examiné le rôle de la régulation endocannabinoïde des neurones dopaminergiques sur le comportement alimentaire.

Méthodes: Le domaine 6 des enzymes alpha / bêta-hydrolase de la sérine hydrolase (ABHD6) dégrade le eCB 2-arachidonoylglycérol, contrôlant ainsi ses actions de signalisation au niveau du récepteur cannabinoïde 1. Nous avons cherché à supprimer de manière conditionnelle l'ABHD6 via des microinjections au niveau du VTA d'un AAV délivrant Cre (ou un contrôle de la GFP) sous le contrôle d'un promoteur spécifique aux neurones (Syn) ou d'un promoteur spécifique aux neurones dopaminergiques (Th). Trois semaines après la convalescence chirurgicale, les souris ont été soumises à un jeûne de 22 heures, après quoi le dosage de nourriture a été mesuré, avec un suivi de l'alimentation et du poids corporel sous un régime de nourriture normale (chow diet) ou sous un régime riche en graisses (high-fat diet). Un autre groupe de souris a été entraîné pour répondre à des granules riches en graisses et en saccharose lors d'une tâche opérante à rapport progressif, et les microinjections suivantes ont été testées pour rechercher des modifications dans la réponse opérante.

Résultats: Les injections d'un virus Th.Cre au niveau du VTA ont augmenté la prise alimentaire cumulative d'un régime riche en graisses (mais pas de nourriture normale) par rapport au contrôle, sans affecter les réponses à la réalimentation. De plus, une tendance à l'augmentation de la réponse opérante dans une tâche opérante a été observée chez des souris recevant des injections de Th.Cre à la suite d'un jeûne de 22 heures.

Conclusions: Ces résultats suggèrent que le système endocannabinoïde joue un rôle dans la régulation de la prise d'aliments palatables par les neurones VTA de la dopamine, et éclaireront les expériences futures sur le rôle de la signalisation de l'eCB sur le comportement dépendant de la dopamine.
Objectif : La pratique d’activité physique (AP) régulière est liée à plusieurs bénéfices pour la santé chez les personnes atteintes de diabète de type 1 (DbT1), incluant l’amélioration de la santé cardiovasculaire et de la qualité de vie. Toutefois, plus de 60 % des patients avec le DbT1 sont sédentaires, ce qui contribue à augmenter les facteurs de risque cardiométabolique. L’augmentation du risque d’hypoglycémie est la barrière la plus importante rapportée par les patients pour la pratique de l’AP. La principale cause de ce risque d’hypoglycémie est l’incapacité des personnes atteintes du DbT1 à réduire le niveau d’insuline plasmatique durant l’AP, contrairement aux individus sans DbT1. Un apport en glucides est souvent requis pour prévenir les hypoglycémies lors d’une AP prolongée. La quantité de glucides nécessaire peut varier selon le type d’insulinothérapie, le moment où l’AP est effectuée, le type d’AP et la glycémie au début de l’AP. En plus de la quantité de glucides ingérée, le moment où les glucides sont consommés pourrait également avoir un impact sur le contrôle de la glycémie durant l’AP. Une prise de glucides à un moment unique avant une AP pourrait induire une hyperglycémie transitoire, alors que la même quantité de glucides répartie durant l’AP pourrait être associée à un meilleur profil glycémique. Notre objectif est de comparer l’efficacité de 2 stratégies de collation pour maintenir la glycémie dans les cibles glycémiques (4,0 à 10,0 mmol/L) lors d’une AP chez les adolescents et adultes avec le DbT1 traités par multi-injections d’insuline.

Méthodes : Trois heures et demie après avoir consommé leur diner standardisé, les sujets ont participé à 2 interventions durant lesquelles une heure de vélo stationnaire est réalisée à 60% de leur VO2peak. Aléatoirement, ils consommaient un apport en glucides de 0,5g/kg, soit en une seule fois 5 minutes avant l’AP (stratégie de prise unique) ou répartie avant et pendant l’AP (40% 5 minutes avant l’AP, 30% à la 20ème minute et 30% à la 40ème minute) (stratégie de prise répartie). La glycémie capillaire est mesurée avant l’AP, puis toutes les 10 minutes durant l’AP.

Résultats : Vingt-six des 33 participants planifiés ont complété l’étude. Il y a eu 3 épisodes d’hypoglycémie (< 4,0 mmol/L) qui sont survenus durant l’AP pour la stratégie de prise unique et 4 pour la stratégie de prise répartie. La glycémie de départ moyenne était de 7,9 mmol/L avec la stratégie de prise unique et de 7,5 mmol/L avec la stratégie de prise répartie. Le pourcentage du temps passé dans les cibles glycémiques était de 70 ± 37% avec la stratégie de prise unique et de 88 ± 26% avec la stratégie de prise répartie (p=0,02). Le pourcentage du temps passé au-dessus de 10 mmol/L était de 23 ± 36% avec la stratégie de prise unique et de 9 ± 26% avec la stratégie de prise répartie (p=0,05). En moyenne, la glycémie des sujets a diminué de 1,34 ± 1,33 mmol/L avec la stratégie de prise unique comparativement à une diminution de 0,54 ± 0,72mmol/L avec la stratégie de prise répartie (p=0,005).

Conclusion : Nos résultats préliminaires suggèrent que les deux stratégies de collation sont similaires pour prévenir les hypoglycémies lors de l’AP. Cependant, la stratégie d’apport en glucides consommé de façon répartie pourrait être associée à un meilleur profil glycémique.
21 - Genetic disruption of Adipose Triglycerides Lipase (ATGL) in mediobasal hypothalamic neurons induces overweight and metabolic disturbances

Romane Manceau\textsuperscript{1,2}, Sébastien Audet\textsuperscript{1,2}, Arturo Israel Machuca-Parra\textsuperscript{1}, KHALIL BOUYAKDAN\textsuperscript{1,2}, Demetra Rodaros\textsuperscript{1}, Alexandre Fisette\textsuperscript{2}, Grant Mitchell\textsuperscript{3}, Stephanie Fulton\textsuperscript{1,2}, Thierry Alquier\textsuperscript{1,2}

\textsuperscript{1}CRCHUM, \textsuperscript{2}Université de Montréal, \textsuperscript{3}Ste-Justine Hospital

Background: Adipose Triglyceride Lipase (ATGL) acts as the first lipase in the hydrolysis of triglycerides (TG). Recent studies show that ATGL in peripheral tissues plays major roles on energy homeostasis. We found that ATGL is expressed in the mediobasal hypothalamus (MBH) and in hypothalamic neuronal cell lines, in line with our recent study suggesting that neurons accumulate TG. ATGL expression is increased in the MBH of high fat-fed mice that maintain a healthy body weight compared to mice that become obese. In addition, ATGL expression in the MBH is increased in response to fasting. This suggests that increased ATGL may play a role in maintaining a healthy metabolic profile. We propose that hypothalamic ATGL regulates lipid metabolism in the brain that in turn contributes to energy balance.

Materials and methods: To test this hypothesis, synapsin-Cre or -GFP expressing AAV are stereotaxically injected in the arcuate nucleus (ARC) of male ATGL flox mice to KO ATGL specifically in neurons (ATGLKO).

Results: First, we validated that ATGL expression is reduced by 50% in ATGLKO mice. We found that ATGLKO have increased weight gain on a chow diet compared to control animals that is associated with reduced energy expenditure and increased food intake and fat mass. In addition, chow-fed ATGLKO mice have an increased fasting glycaemia and mild glucose intolerance. Finally, pharmacological inhibition of ATGL in hypothalamic neurons in vitro increases intracellular TG content.

Conclusion: Together, our findings suggest that the ATGL pathway in MBH neurons beneficially regulates glucose and energy homeostasis by mechanisms that may involve regulation of TG and lipid droplets metabolism.
22 - Leptin receptor expression and the blood brain barrier (BBB)

Liliia Butiaeva¹, Sarah Robins¹, Tal Slutzki¹, Xiaohong Liu¹, Maia Kokoeva¹

¹McGill University, Department of Medicine, Division of Endocrinology and Metabolism

Previous studies suggest that brain circuits controlling energy balance can undergo morphological changes affecting dendritic spine formation and arbour length upon dietary challenge. However, such changes have yet to be demonstrated directly in individual cells over time. To this end, we employed optical microendoscopy to monitor individual, fluorescently labeled leptin receptor (LepR) cells in the mediobasal hypothalamus (MBH) in living mice. Time-lapse, intravital fluorescent imaging, in conjunction with co-labeling of the vasculature by intravenously delivered fluorescent dextran, revealed an unexpected finding that some LepR-processes are tightly associated with micro-capillaries. We confirmed these results in slice preparations of intact, non-implanted mice ruling out that the observed alignment is a surgery artefact. Furthermore, we discovered that some LepR-positive cells are not neurons. We identified the nature of the cells using their morphology, location and markers expression. Importantly, we showed that LepR-KO specifically in identified cell type suggested a role of these cells in energy balance, and examined the effect of EPAC agonists/antagonists on blood vessel permeability and energy balance. Collectively, our data are the first to demonstrate structural plasticity within individual neurons of the MBH energy balance circuitry in response to physiologically relevant cues, and to reveal previously undescribed cell type with LepR expression and a role of these cells in energy balance regulation.
23 - Inhibition of ATGL reduces inflammation in LPS-activated microglial cells

Arturo Israel Machuca-Parra¹, Romane Manceau¹, Demetra Rodaros¹, Cyril Laurent¹, Nathalie Arbour¹, Stephanie Fulton¹, Thierry Alquier¹

¹CRCHUM, CHUM, Université de Montréal, QC, CA

Microglia, the intrinsic immune system of the brain, is the primary cell population responding to insults that alter brain homeostasis generating different molecular and morphological profiles: the classically activated M1-type and the alternatively activated state M2-type. During inflammation, large lipid droplets (LDs) are actively formed and constitute sites for the synthesis and storage of various inflammatory mediators in many cell types, including neurons and microglia. Specific glycerolipid lipases surrounding LDs catalyze the hydrolysis of triglycerides (TG) to generate mono-(MAG), di-acylglycerol (DAG), and fatty acids (FA). The Adipose Triglyceride Lipase (ATGL), which catalyzes the hydrolysis of triglycerides, plays a key role in lipid homeostasis by regulating glycerolipid metabolism in several cell types. However, the role of ATGL and LDs in microglial cell function and neuroinflammation is unknown.

We found that ATGL is enriched in FACS-purified microglial cells from adult mouse brain and is expressed in postnatal microglial cells in culture. Primary microglia cultures derived from mouse pups (P2) and BV2 cells were treated with Atglistatin (a specific ATGL inhibitor) to determine the effect of ATGL inhibition on apoptosis and cell viability measuring cell health and viability, and pro-inflammatory responses induced by LPS. The expression of different inflammatory markers was measured by qRT-PCR.

A significant decrease in apoptosis (p<0.0002) and cytotoxicity (p<0.0004) with Atglistatin was observed. ATGL expression was decreased (p<0.001) in the presence of LPS. Expression levels of IL-6 (p<0.0001) and MCP-1 (p<0.0001) were decreased in the presence of Atglistatin under inflammatory conditions but not for IL-1b, TNF-a and NF-kB. With ORlistat, a general lipase inhibitor, expression levels of IL-1b (p<0.0001), IL-6 (p<0.0001), and NF-kB (p<0.0001), but not MCP-1 and TNF-a, were significantly decreased under inflammatory conditions.

We propose that ATGL regulates inflammatory responses in LPS-stimulated microglia, suggesting a significant role of ATGL during inflammation in vitro. Ongoing experiments are aimed at assessing the role of ATGL in TG metabolism, LDs dynamics and inflammation processes in vivo using genetic loss-of-function models.
Novel glycerol-3-phosphate phosphatase/PGP-like homologs are implicated in metabolism, stress responses and lifespan in *C. elegans*.

Elite Possik\(^1,2\), Clemence Schmidt\(^1,2\), Johanne Morin\(^1,2\), Heidi Erb\(^1,2\), Anfal Almass\(^1,2\), Wahab Kohlan\(^1\), Alex Parker\(^2,3\), SR Murthy Madiraju\(^1,2\), Marc Prentki\(^1,2\)

\(^1\)Department of Nutrition, Montreal Diabetes Research Center, CRCHUM, 900 St-Denis, \(^2\)CRCHUM, \(^3\)Department of Neurobiolog, CrCHUM

Recently, Prentki Lab has identified a novel enzyme in mammalian cells, glycerol-3-phosphate phosphatase (G3PP), also called phosphoglycolate phosphatase (PGP), capable of hydrolyzing glycerol-3-phosphate, a central metabolic intermediate, to glycerol. This is of great interest for intermediary metabolism at large and possibly also for cardiometabolic disorders, since activation of this enzyme could divert excess glucose, that can be toxic to cells, to form more innocuous glycerol. The function and role of G3PP in vivo in various organisms is poorly understood.

We used the nematode *C. elegans* as a model system to study the metabolic and biological functions of G3PP. Using blast alignment, we identified three PGP-like homologs/G3PP genes in *C. elegans*, K09H11.7, C53A3.2, and F44E7.2. We found that G3PP transcripts are upregulated during hyperosmotic stress and glucose feeding in *C. elegans*. Importantly, glycerol accumulates in animals exposed to hyperosmotic stress and high glucose levels in a G3PP-dependent manner, supporting a role of G3PP in the protection from hyperosmotic stress and glucotoxicity. To elaborate the role of G3PP in vivo, we generated double and triple G3PP deletion mutant animals using Crispr-CAS9 technology. Loss of G3PP decreased resistance to hyperosmotic and metabolic stresses, and exacerbated glucotoxicity and shortened lifespan. Importantly, G3PP mutant animals at younger age do not show physiological defects (brood size, egg laying, locomotion, exercise, and pharyngeal pumping), indicating that loss of G3PP is unlikely to cause adverse metabolic toxicity under normal growth conditions. However, the data demonstrate an age-dependent decline in behavior in movement and locomotion supporting an essential role of this enzyme in organismal aging. Moreover, loss of G3PP also increases fat content in *C. elegans* supporting a role for G3PP in the regulation of metabolism. Moreover, we prepared transgenic strains overexpressing the three G3PP/PGP-like enzyme and human G3PP/PGP in the worm model and we are currently assessing whether elevated activity of this enzyme extends lifespan and enhances survival to various stresses. Metabolomics studies using the mutant G3PP enzymes and overexpressors will be conducted to help decipher G3PP roles in metabolism. Importantly, we recently identified ELT-2/GATA4 as a major transcription factor that regulates G3PP expression in the worm, using an RNAi screen strategy designed to suppress transcription factor genes in G3PP transcriptional reporter *C. elegans* strains. Overall, the current data suggest that G3PP/PGP is an evolutionary conserved regulator of glucose and fat metabolism that act to protect against nutrient and environmental stresses and is possibly involved in healthy aging.
25 - A link between early-life exposure to environmental pollutants and diabetes risk

Myriam Hoyeck\textsuperscript{1}, Jenny Bruin\textsuperscript{1}

\textsuperscript{1}Carleton University

Diabetes prevalence is increasing at exponential rates, and epidemiological studies have shown a correlation between pollutant exposure and diabetes incidence. Dioxins are a group of highly persistent organic pollutants that show widespread global distribution. Preliminary data in the Bruin lab has shown that exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most toxic dioxin, upregulates Cyp1a1 expression (a biomarker of dioxin exposure) in islets, suppresses glucose-stimulated insulin secretion in human and mouse adult β-cells, and causes β-cell loss in adult mice. Abnormalities in β-cell mass and function are key characteristics of diabetes, as such TCDD exposure may pre-dispose females to diabetes-associated complications during pregnancy. In addition, studies have shown that dioxins cross the placenta and are excreted in breast milk; therefore, it is possible that maternal exposure to dioxins could alter pancreas development and function in offspring and may confer a lifetime risk of developing diabetes. This study analyzed the effects of chronic low-dose TCDD exposure to dams during gestation and lactation on pancreas development and long-term diabetes risk in both male and female offspring, as well as effects on dam metabolism. Female mice were treated with TCDD (20 ng/kg/d) or corn oil (vehicle) 2x per week prior to and throughout gestation, and during lactation until weaning. Plasma, liver, and pancreas were collected from offspring at birth and weaning (3 weeks of age) for analysis by ELISA, qPCR, and immunohistochemical staining. A subset of offspring was transferred to a high fat diet (HFD) at 12 weeks of age (9 weeks after TCDD exposure ceased), and a subset of dams received HFD beginning at 8 weeks post-exposure. Long-term changes in pancreas function were assessed pre- and post-HFD using in vivo glucose-stimulated insulin secretion assays, and glucose and insulin tolerance tests. Neonates from TCDD-treated dams had significantly decreased blood glucose levels and increased plasma insulin levels compared to control offspring at birth. The decrease in blood glucose persisted until 3 weeks. At 6-9 weeks, TCDD-exposed males were modestly hyperglycemic during a glucose tolerance test and significantly insulin resistant, whereas female offspring did not display lasting effects on glucose homeostasis. Interestingly, TCDD exposure during pregnancy did not have lasting effects on blood glucose or insulin secretion in the dams, but did promote increased weight gain relative to control dams starting approximately 5 weeks post-exposure. The effect of HFD on TCDD-exposed offspring and dam pancreas function is currently being assessed. These results suggest that early-life exposure to TCDD may predispose male offspring to defects in pancreas function and increased diabetes risk. Additionally, TCDD exposure during pregnancy promotes excessive weight gain in dams. Taken together, our data supports epidemiological evidence that pollutant exposure may be a causal factor driving diabetes risk.
26 - Beta-cell compensation to pubertal insulin resistance is compromised in high-fat fed rats and impairs glucose homeostasis later in life

Anne-Laure Castell¹,², Mélanie Ethier², Grace Fergusson², Julien Ghislain², Vincent Poitout¹,²

¹Université de Montréal, ²CRCHUM

Background: Puberty is a time of hormonal changes that are associated with insulin resistance (IR). Although insulin sensitivity is restored at the end of puberty in healthy youth, it does not resolve in obese adolescents leading to an increased risk of metabolic disease such as type 2 diabetes. In pregnancy and obesity-induced IR, β-cells increase their functional mass to maintain glucose homeostasis. During puberty, however, the mechanism of pancreatic β-cell compensation to IR and its role in glucose metabolism later in life have not been established.

Objective: To characterize pancreatic β-cell adaptation to pubertal IR in rats and study the effect of metabolic stress during puberty on glucose homeostasis in adult animals.

Methods: Male and female Wistar rats were fed a chow diet or a high fat diet (HFD) during puberty. Body weight, fasted plasma insulin, glucose tolerance and hormone levels (estradiol, testosterone, insulin growth factor-1 (IGF1), growth hormone (GH)) were determined every 5 days from weaning to adulthood. β-cell proliferation was assessed by immunostaining of pancreatic cryosections for Ki67 and insulin to mark β-cells and β-cell mass by morphometric analysis of insulin staining.

Results: During puberty, glucose intolerance was associated with an increase in insulin levels in both sexes, suggestive of IR. Correspondingly, β-cell proliferation increased, as did islet size and β-cell mass. β-cell expansion correlated with a rise in IGF1/GH levels. HFD during puberty impaired glucose tolerance in adults.

Conclusion: During puberty in rats, β-cells compensate for increased IR. Metabolic stress during puberty impairs glucose homeostasis later in life. Future studies will address whether β-cell expansion during puberty is under control of the IGF1/GH axis.
27 - BMP9 (Bone Morphogenetic Protein-9) non-canonical signaling Akt/Fox01 inhibits hepatic gluconeogenesis via Alk3

Akla N1,2,5,6, Sapieha P1,2,4,5,6, Larrivée B1,3,4,5,6

1Université de Montréal, 2Département de biochimie et médecine moléculaire, 3Département des sciences biomédicales, 4Département d'ophtalmologie, 5Centre de recherche de l'Hôpital Maisonneuve-Rosemont, 6Faculté de Médecine de l'Université de Montréal

Rationale: A subfamily of the transforming growth factor (TGF)-beta family, Bone morphogenetic proteins (BMPs) regulate an array of cellular functions during development and in the adult. Secreted by the liver, circulating BMP-9 potently induces osteogenesis, has been implicated in vessel stability, and recent data suggest that it regulates glucose metabolism. However very little is known about the mechanisms behind the effects of BMP9 on glucose homeostasis. This study aims to investigate BMP9 effect on hepatic gluconeogenesis and its mechanisms.

Methods: The regulatory effects of BMP9 overexpression were investigated in control and STZ diabetic mice. Blood glucose levels were monitored in STZ mice for 4 weeks following BMP9 adenodelivery and assayed for glucose control with fructosamine. Glucose metabolism was evaluated by glucose, pyruvate and insulin tolerance tests. The effects of BMP9 on glycogenesis and hormonal levels of corticosteroid and glucagon where also measured. Experiments were performed on primary hepatic cells using WB and siRNA silencing to assess the mechanistic basis of BMP9 on glucose homeostasis.

Results: A single dose of hepatic adenoviral vector overexpressing BMP9 in diabetic mice resulted in lower blood glucose to near normal levels and improved their glucose control over a span of 4 weeks after treatment. BMP9 decreased hepatic glucose production through inhibition of gluconeogenesis and a decrease in transcription and expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase). We found that BMP9 regulates expression of gluconeogenic enzymes through activation of AKT at Ser473, which promoted FoxO1 phosphorylation and inactivation. BMP9 type 1 receptors siRNA invalidation showed that BMP9 acts via Alk3 in mediating AKT/Fox01 activation and gluconeogenic enzyme inhibition. This effect seems to be independent of insulin action or levels.

Conclusion: Together, these data indicate that BMP9 overexpression in the liver improves glucose control in type I induced diabetic mice, independently of insulin. We show that BMP9 reduces hepatic glucose output through Alk3/AKT/Fox01 signaling and inhibition of gluconeogenic enzymes. Hence BMP9 hepatic signaling would serve as a new therapeutic approach in the treatment of diabetes and its related metabolic alteration involving insulin resistance.
Defining the cellular mechanisms of DPP4 shedding

Branka Vulesevic1,2, Natasha Jeraj 1, Natasha Trzaskalski2, Erin Mulvihill1,2

1University of Ottawa, 2University of Ottawa Heart Institute

Dipeptidyl peptidase 4 (DPP4) is a widely expressed, serine protease that cleaves and inactivates a number of proteins including the incretin hormones glucagon-like peptide-1 and glucose-dependent insulinoikotropic polypeptide. In response to food consumption, the incretin hormones are secreted from the gut and potentiate meal-stimulated insulin secretion. DPP4 inhibitors successfully regulate glucose levels in patients with Type 2 Diabetes Mellitus (T2DM). DPP4 exists in two enzymatically active isoforms - a membrane-bound form and a circulating, soluble form. High levels of soluble DPP4 are associated with obesity, T2DM and non-alcoholic fatty liver disease (NAFLD). It has been proposed that circulating DPP4 may originate from the liver in response to high-fat diet-induced lipid accumulation. Sheddases (Mmps/Adams) have been implicated in the release of DPP4 from the membrane in response to lipid accumulation and inflammation. Here, to better understand the molecular underpinnings of DPP4 shedding, we studied the effects of lipid accumulation, specifically in the liver, on the expression of candidate sheddases. Liver samples were obtained from wild-type and Pemt-/-mice, a model of NAFLD. Pemt-/- mice had a 3-fold elevating in circulating DPP4 concentrations. Concurrently, we observed that increased hepatic lipid accumulation resulted in upregulation of sheddases, specifically Adamst, Mmp2, and Mmp9. However, further evaluation of Mmp9-/- mice and littermate controls demonstrated no difference in plasma DPP4 activity suggesting it is not a key factor in DPP4 release. Additionally, we set out to understand the role of lipid loading and inflammation, a common feature of metabolic syndrome, in DPP4 release from hepatocytes. We demonstrate that treatment of hepatocytes with inflammatory cytokines or fatty acids is not sufficient to induce DPP4 release. Understanding the pathways that regulate DPP4 cleavage and shedding is necessary to improve current therapies for patients with metabolic syndrome. Understanding the molecular mechanism of DPP4 shedding will provide insight into the predictive value of soluble DPP4 as a biomarker of NAFLD.
29 - Defining the short-term effects of pharmacological 5’-AMP activated kinase modulators on kidney cell mitochondria

Dana Abou Samhadaneh¹, Ossama Moujaber², Ursula Stochaj³

¹McGill University, ²McGill University, ³McGill University

Background. Within the kidney, proximal tubules require large energy supply for active transport of organic molecules and ions. This is predominantly provided by mitochondria, which makes proximal tubules especially vulnerable to organelle dysfunction, such as diabetic nephropathy. 5’-AMP activated kinase (AMPK) functions as an energy sensor and is implicated in numerous biological processes. This kinase provides a focal point for metabolic control in all eukaryotes. Different pharmacological AMPK activators have been used to overcome the loss of proper mitochondrial function. As a crucial regulator of glucose, lipid and protein homeostasis, AMPK is an important therapeutic target for type 2 diabetes, obesity and cancer. Long-term treatment with activators of AMPK enhances mitochondrial biogenesis. However, their short-term effects on mitochondria and impact on healthy cells is not well understood.

Methods. We evaluated the impact of four pharmacological AMPK modulators (compound C, phenformin, resveratrol, AICAR) on healthy proximal tubule cells of the kidney. Specifically, we quantified the effects on mitochondrial membrane polarization, morphology and heterogeneity using quantitative confocal microscopy and Western blotting.

Results. Short-term incubation with the AMPK inhibitor compound C caused mitochondrial hyperpolarization. This was accompanied by mitochondrial fragmentation. By contrast, AMPK activators AICAR, phenformin and resveratrol had little impact. We further show that the biological properties of mitochondria are determined by their subcellular location. Mitochondria at the cell periphery displayed higher polarization as compared to organelles located in the vicinity of the nucleus. This was not limited to renal proximal tubule cells, but also observed in breast cancer cells. Pharmacological AMPK modulators altered these location-dependent properties in a compound-specific fashion. While the region-dependent differences were enhanced with phenformin, they were ameliorated by resveratrol.

Conclusions. AMPK modulator cause rapid changes in mitochondrial characteristics. Our studies show that pharmacological agents that target AMPK can rearrange mitochondrial networks at the single cell level. These insights are relevant to the development of proper strategies for the short-term adjustment of mitochondrial performance.
30 - Deletion of 14-3-3ζ in pancreatic β-cells potentiates glucose-stimulated insulin secretion

Yves Mugabo1,2,3, Assétou-Aïcha Konaré1,2, Gareth Lim1,2,3

1CRCHUM, Montréal, QC H2X 0A9, Canada, 2Department of Medicine, Université de Montréal, QC H3T 1J4, Canada, 3Montreal Diabetes Research Center, Montreal, QC H2X 0A9, Canada

Pancreatic β-cells continuously sense levels of blood sugar and secrete insulin to maintain normoglycemia. Aberrant insulin secretion contributes to various disorders such as diabetes and cardiometabolic diseases. In β-cells, ATP couples glucose sensing to insulin granule exocytosis, whereby it triggers closure of ATP-sensitive potassium channels to promote membrane depolarization, increases in intracellular calcium, and ultimately insulin secretion. 14-3-3 proteins, and in particular 14-3-3ζ, have been found to regulate ATP synthase and mitochondrial respiration, suggesting that they may regulate glucose-stimulated insulin secretion (GSIS). Thus, we hypothesized that 14-3-3 proteins may regulate mitochondrial function in β-cells and GSIS.

Through ex-vivo and in vivo experiments, we have identified critical roles of 14-3-3 proteins and 14-3-3ζ in GSIS. Pan-inhibition of 14-3-3 proteins with 14-3-3i,2-5 and BV02 (10µM) in mouse islets potentiated ex-vivo GSIS, which correlated with glucose-dependent respiration and ATP production, as determined by Seahorse Extracellular flux measurements.

Of the seven isoforms, we previously reported critical roles of 14-3-3ζ in glucose homeostasis and metabolism, and to understand its role in β-cells, we generated β-cell specific knockout mice (Ins1CreThor:14-3-3ζfl/fl, β-KO). When compared to wild-type (WT) littermate controls, β-KO mice did not display differences in body weight or defects in glucose tolerance; however, after an overnight fast, β-cell-specific deletion of 14-3-3ζ significantly enhanced insulin secretion following i.p. glucose (2 g/kg). Levels of Ins2 and Pdx1 mRNA were significantly elevated in islets from β-KO mice, assessed by quantitative PCR. Mitochondrial function was measured in isolated islets from WT and β-KO mice, and significantly increased ATP production and oxygen consumption rates in β-KO islets were detected.

Collectively, these results suggest that 14-3-3 proteins, and in particular 14-3-3ζ, have important roles in GSIS, likely through effects on mitochondrial ATP production. As there are seven mammalian isoforms, it will be interesting to assess the roles of other 14-3-3 protein members in the regulation of GSIS. However, based on these data, it suggests that 14-3-3ζ could have predominant roles. Additionally, these data suggest that 14-3-3ζ inhibition may represent a promising target to enhance pancreatic β-cell function.
31 - Etude de l’interaction de Nck1 et PERK dans la fonction et la survie des cellules beta pancréatiques

Emilie Courty1,2, George Kefalas2,3, Nathalie Jouvet1, Cindy Baldwin1, Louise Larose2,3, Jennifer Estall1,2

1Institut de recherches cliniques de Montréal, 2McGill university, 3Research Institute of the McGill University Health Center

Les diabètes de type 1 et 2 sont caractérisés par un défaut de sécrétion d’insuline par les cellules bêta pancréatiques. Maintenir une masse fonctionnelle de cellule bêta est donc un enjeu thérapeutique dans le traitement du diabète. La mutation de la protéine PERK entraîne un diabète néonatal et a été impliquée dans le maintien du repliement correct de l’insuline. Il a été mis en évidence que la protéine adaptatrice Nck1 régulait négativement l’activité de PERK et que son invalidation entraînait une augmentation du contenu en insuline (Yamani et al 2014) et protégeait les cellules bêta de l’apoptose dans des conditions gluolipotoxiques (Yamani et al 2015).

Dans le but d’étudier spécifiquement l’interaction de Nck1 et PERK dans les cellules bêta ; nous avons créé un phospho-peptide possédant la séquence de PERK et capable de lier Nck1. Des cellules INS-1 ont été cultivées en conditions gluco-lipotoxiques (palmitate 2mM et glucose 25 mM) et été traitées en présence ou non du peptide (10uM) pendant 3 jours. La survie ainsi que la fonction de ces cellules ont été étudié. Des ilots humains de patients contrôles et DT2 ont également été traités en présence ou non du peptide et un test de sécrétion d’insuline en réponse au glucose ont été réalisé.

Les cellules INS-1 cultivées en condition gluco-lipotoxiques et traitées avec le peptide présentent une diminution de l’apoptose associée à une augmentation de la sécrétion d’insuline en réponse au glucose comparé aux cellules non traitées avec le peptide. De manière très intéressante les ilots humains de DT2 présentent également une augmentation du contenu et de la secretion d’insuline en réponse au glucose lorsqu’ils sont cultivés en présence du peptide tandis que le peptide n’a aucun effet sur les ilots de patients sains.

Ces résultats mettent en évidence qu’empêcher l’interaction pharmacologique de Nck1 avec PERK améliore la fonction et survie des cellules bêta dans des conditions diabétogènes. L’administration de ce peptide dans des modèles animaux de diabète constitue une piste prometteuse dans la protection des cellules bêta.
HB-EGF signaling is required for glucose-induced pancreatic β-cell proliferation in rats

Hasna Maachi¹,²,³, Mallikarjuna R. Metukuri⁴, Donald Scott⁴, Julien Ghislain¹,², Vincent Poitout¹,²,³

¹Montreal Diabetes Research Center, ²CRCHUM, ³Department of Medicine, University of Montreal, ⁴Icahn School of Medicine at Mount Sinai, NY, USA

Background: Glucose is a major β-cell mitogen. Despite recent progress, the underlying mechanisms remain unclear. In a rat model of nutrient excess we previously showed that nutrient-induced β-cell proliferation is blocked when either EGF receptor (EGFR) or mTOR signalling is inhibited. Parallel transcriptomic analyses identified the EGFR ligand, HB-EGF as a potential mediator of nutrient-induced β-cell proliferation.

Objective: To determine the role of HB-EGF in glucose-induced β-cell proliferation.

Methods: HB-EGF mRNA levels were assessed by real-time PCR in isolated rat islets following a 24-h exposure to 2.8 or 16.7 mM glucose. The Carbohydrate-Responsive Element-Binding Protein (ChREBP) transcription factor was down-regulated by siRNA in dispersed rat islets. For β-cell proliferation studies islets were exposed to 16.7 mM glucose or HB-EGF (100 ng/ml) in the presence of 2.8 mM glucose for 72 h. Islets were co-cultured with the EGFR inhibitor AG1478 (300 nM) or the HB-EGF inhibitor CRM197 (10 ug/ml). shRNA was used to knockdown HB-EGF in isolated islets and either cultured ex vivo or transplanted under the kidney capsule of glucose-infused rats. β-cell proliferation was assessed by immunohistochemistry for Ki67 and insulin.

Results: Glucose increased HB-EGF mRNA levels and this was prevented by ChREBP knockdown. HB-EGF potently stimulated β-cell proliferation. Inhibition of the EGFR or HB-EGF completely blocked not only the proliferative response to HB-EGF but also the response to 16.7 mM glucose. Knockdown of HB-EGF blocked the β-cell proliferative response to glucose in isolated rat islets as well as in transplanted islets.

Conclusion: HB-EGF is a potent β-cell mitogen in rat islets. Glucose increases HB-EGF gene expression via ChREBP. The proliferative response to glucose requires an intact HB-EGF – EGFR pathway. Our findings identify a novel player in the complex mechanisms controlling β-cell proliferation in response to glucose. (NIH, FRQS)
33 - Identification de facteurs sécrétoires dépendants de l'expression hépatique de PGC-1a dans un contexte de développement de la stéatose hépatique non alcoolique.

Philipa Levesque-Damphousse¹,², Aurèle Besse-Patin¹,²,³, Wulan Chen⁴, Philippe Besse⁵, Jennifer L Estall¹,²,³

¹Université de Montréal, ²Institut de recherches cliniques de Montréal, ³McGill University, ⁴McMaster University, ⁵Institut de Mathématiques de Toulouse

La stéatose hépatique non alcoolique (SHNA) regroupe un ensemble de maladies du foie et est caractérisée par une accumulation de triglycérides dans plus de 5% des hépatocytes. Cependant, les mécanismes physiopathologiques de la maladie sont peu connus. Sa prévalence croissante nous pousse à étudier son développement et à trouver des méthodes de diagnostic précoces et non-invasives. Le laboratoire de Dre Estall a montré que le niveau d'expression du coactivateur transcriptionnel PGC-1a corrèle avec la sévérité de la stéatose hépatique, le stress oxydatif et la résistance à l'insuline dans les foies de souris. Chez l'humain, on observe aussi une diminution de PGC1-a dans les foies de patients atteints de SHNA. Une réduction de 50% de PGC-1a dans les foies murins (LH) mène à une insensibilité à l'insuline et à une tolérance au glucose altérée dans les tissus périphériques. Ces découvertes suggèrent que PGC-1a affecte les protéines sécrétées par le foie et ainsi influence la régulation métabolique de tout le corps.

Objectif: Identifier des facteurs sécrétés par le foie dont l'expression corrèle avec les niveaux hépatique de PGC-1a et donc d'identifier de potentiels biomarqueurs de stéatose hépatique non alcoolique. Méthode: À l'aide de protéomique quantitative, nous identifions des facteurs sécrétés par des hépatocytes primaires isolés de souris sauvages (WT) ou souris hétérozygotes (LH) nourris avec une diète riche en gras/fructose (HFHF) ou diète contrôle (CHOW). Les niveaux sanguins et hépatiques de ces protéines seront quantifiés et leurs effets sur les tissus périphériques seront analysés. Résultats: Nous avons identifié SerpinA3N, dont l'expression protéique et génique est altérée par la combinaison de la diète HFHF et de la réduction hépatique de PGC-1a (LH). SerpinA3N est un inhibiteur de protéase à sérine et des résultats préliminaires montrent que les niveaux hépatiques et sanguins de SerpinA3N augmentent chez les jeunes souris obèses. Conclusion: Ce projet permettrait d'identifier SerpinA3N comme potentiel biomarqueur de faible niveau hépatique de PGC-1a et de stade précoce de la stéatose hépatique non alcoolique.
34 - Identification of a new hepatokine expressed and secreted in response to steatosis.

Mathilde Mouchiroud¹, Étienne Camiré¹, Laurie Turcotte¹, Christian Roy¹, Yves Géninas¹, Mathieu Laplante¹

¹Université Laval

Context and objectives: Deregulation in liver metabolism is a major contributor to several obesity-related pathologies including type 2 diabetes, insulin resistance, dyslipidemias and cardiovascular diseases. In response to obesity, the liver develops metabolic disorders leading to non-alcoholic fatty liver disease (NAFLD), a condition that can result in fibrosis, cirrhosis and cancer. In recent years, several proteins secreted by the liver (hepatokines) have been identified. It was shown that some of these proteins have the capacity to modulate the function of peripheral tissues and to contribute to the development of obesity-related diseases. The objective of this research project was to identify and characterize new hepatokines that could be used as biomarkers of liver health or serve as therapeutic targets to improve health and metabolism in obese patients.

Methods and results: We have identified Leucine-Rich Repeat-Containing X (LRRCX) as a secreted protein expressed predominantly in the liver. We found that hepatic expression of LrrcX is elevated in three mouse models of obesity (ob/ob, db/db and diet-induced obese mice). We discovered that the expression of LrrcX strongly associates with hepatic steatosis. Mice fed a methionine-choline free diet, a diet that exacerbates steatosis, showed high hepatic expression of LrrcX and elevated plasma levels of LRRCX. We found a strong link between the expression of LrrcX and endoplasmic reticulum stress, a condition that prevails in fatty livers. Preliminary results with Lrrcxoverexpressing mice reveal a possible role for LrrcX in regulating cholesterol metabolism.

Conclusion: Overall, we report the identification of a new hepatokine expressed and secreted in response to steatosis that could participate in the development of obesity-related metabolic disorders. Further investigations will precise the functions of Lrrcx in metabolism and the mechanisms regulating its expression.
PGC1α is a transcriptional co-activator that regulates genes involved in substrate metabolism and mitochondrial biogenesis and is now linked with anti-inflammatory actions. Hepatic inflammatory signalling can exacerbate fibrogenesis and cell death. We observe that low PGC1α increases pro-inflammatory gene expression, but it is unknown if PGC1α activity is necessary to mount an appropriate inflammatory response. Genetic deletion of liver PGC1α increased circulating alanine aminotransferase after 6 weeks of a methionine-choline deficient diet (MCD), indicating that loss of PGC1α enhances liver damage under inflammatory conditions. The PGC1α gene can express multiple isoforms. Under healthy conditions, only PGC1α1 is detected in liver; however, MCD diet feeding increased PGC1α1 and PGC1α4 at the mRNA and protein level. To examine if PGC1α1 and α4 had overlapping or distinct functions in response to inflammation, we compared gene expression and localisation in hepatocytes overexpressing PGC1α1 or PGC1α4 in the absence and presence of TNF. PGC1α4 is located in the cytoplasm basally and TNF treatment triggered translocation to nuclear regions, while PGC1α1 remained nuclear. Genes related to mitochondrial metabolism, glycolysis and immune function were enriched by PGC1α1. In the absence of TNF, PGC1α4 had little effect on gene expression, but transcriptional activity was enhanced by TNF treatment regulating gene sets involved in the immune response and apoptosis. These results indicate that PGC1α1 and PGC1α4 may have overlapping and distinct roles in the inflammatory response. Thus, we compared the effect of these PGC1α isoforms on cell death in response to TNF. Increased PGC1α1 enhanced markers of cell death, while PGC1α4 attenuated the apoptotic response. PGC1α1 increased NF-κB transcriptional activity, but no changes were detected with high PGC1α4, suggesting the anti-apoptotic effects of PGC1α4 may not act through NF-Kb transcriptional activity. Instead, PGC1α4 increased anti-apoptotic genes BIRC2/3, TNFαip3 in response to TNF and induction of these anti-apoptotic genes was blunted by loss of PGC1α. In conclusion, increases in PGC1α4 may prevent apoptosis in cells responding to an inflammatory challenge.
36 - Role of de novo sphingolipid metabolites in oleate-induced pancreatic β-cell proliferation in rats

Anne Laure Castell¹,²,³, Alexis Vivoli¹,²,³, Valentine Moullé¹,²,³, Julien Ghislain²,³, Vincent Poitout¹,²,³

¹Department of Medicine, University of Montreal, QC, Canada, ²Montreal Diabetes Research Center, ³CRCHUM

Fatty acids (FA) are major regulators of pancreatic β-cell function. In a rat model of nutrient excess we previously showed that FA potentiate glucose-induced β-cell proliferation. Sphingolipids, derived from the intracellular metabolism of FA, act as cellular mediators in the regulation of pancreatic β-cell function. However, the contribution of sphingolipid species to FA-induced b-cell proliferation is unknown.

Objective: To determine the role of de novo sphingolipid synthesis in FA-induced β-cell proliferation.

Methods: Isolated rat islets were exposed to oleate or palmitate (0.5 mM) in the presence of 16.7 mM glucose for 48h. Sphingosine kinase (SphK) 1 and 2 expression was measured by real-time PCR. Serine palmitoyl transferase (SPT), SphK, and the S1P₃ receptor were inhibited with Myriocin, SKI II and TY52156, respectively. β-cell proliferation was assessed in cryosections by immunohistochemical detection of insulin and the proliferation marker Ki67 or by flow cytometry by labeling for C-peptide and EdU incorporation. Wistar rats were infused for 72 hours with glucose and a lipid emulsion (CLI) to induce b-cell proliferation.

Results: The monounsaturated FA oleate, but not the saturated FA palmitate, increased β-cell proliferation. Blocking de novo sphingolipid synthesis by SPT inhibition decreased oleate-induced b-cell proliferation as did inhibition of SphK and S1P₃ receptor activation. Sphk1/2 mRNA levels in islets were not significantly changed following nutrient infusion in rats or FA exposure ex vivo.

Conclusion: β-cell proliferation in response to oleate requires de novo sphingolipid synthesis and S1P₃ signaling. Analyses are underway to assess the contribution of SphK products sphingosine-1-phosphate and dihydrosphinganine-1-phosphate in S1P₃-mediated β-cell proliferation.
37 - Stability of the bone-derived hormone osteocalcin is regulated through its O-glycosylation in mice, but not in human

Al Rifai O1,2, Julien C1, Faubert D1, Ferron M1,2

1Institut de recherche clinique de Montréal, Université de Montréal, 2Faculté de Médecine, Université de Montréal

Introduction: Osteocalcin (Ocn) is a bone-derived hormone that regulates glucose and energy metabolism. Ocn is secreted specifically by osteoblasts, the bone forming cells. Our laboratory has shown that before it is secreted by osteoblasts the Ocn precursor (pro-Ocn) undergoes at least two different post-translational modifications (PTMs) which regulates its endocrine functions. First, Ocn is γ-carboxylated on three glutamic acid residues by the γ-carboxylase (GGCX) through a reaction that requires reduced vitamin K as cofactor. Genetics, cellular and clinical studies support the notion that carboxylation inhibits Ocn endocrine function in mouse and human. Second, we have recently shown that the proprotein convertase (PC) furin is critical to convert the inactive pro-Ocn into mature active Ocn. Interestingly, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analysis of Ocn purified from primary mouse osteoblasts supernatant reveals that this protein is subjected to O-glycosylation. In addition, we found that mutation of serine 57 to alanine (S57A) totally abolishes Ocn O-glycosylation in osteoblasts. However, the impact of this PTM on Ocn endocrine function and whether it regulates its γ-carboxylation or processing by furin are still unknown.

Hypothesis: Ocn O-glycosylation regulates its endocrine functions.

Methods and results: The apparent molecular weight of Ocn is reduced when expressed in CHO-Ldld cells, which are defective in O-glycosylation due to a deficiency in UDP-Gal/UDP-GalNAc 4-epimerase, as compared to its expression in parental CHO cells. Supplementation with N-acetylgalactosamine and galactose blunted this difference, confirming that Ocn is indeed O-glycosylated in these cells. Treatment of bone homogenate of wild type mice with O-glycosidase and neuramidase also decreases Ocn apparent molecular weight, suggesting that Ocn is O-glycosylated in vivo. Quantitative analysis of the mRNA expression of N-acetylgalactosamine transferase (GalNAc-Ts), the enzymes involved in O-glycosylation, reveals that GalNAc-T1, 2, and 3 are highly expressed in osteoblasts. The deletion of these GalNAc-Ts in HEK293 partially inhibits Ocn O-glycosylation suggesting that multiple GalNAc-Ts can redundantly glycosylate Ocn. The pharmacological inhibition of GalNAc-Ts in osteoblasts does not affect Ocn processing and γ-carboxylation, while Ocn is still O-glycosylated in osteoblasts when γ-carboxylation and processing are inhibited. Thus, Ocn O-glycosylation occurs independently of its γ-carboxylation and processing. Furthermore, our data shows that Ocn O-glycosylation increases its half-life in plasma in vitro and in vivo in Ocn-/- mice compared to recombinant deglycosylated Ocn produced in bacteria. Interestingly, human Ocn does not appear to be O-glycosylated when expressed in CHO or in osteoblasts. Its amino acid alignment with mouse Ocn shows that the residues corresponding to serine 57 is a tyrosine in the human protein. Mutation of this tyrosine to a serine results in human OCN O-glycosylation when expressed in mammalian cells. Moreover, O-
glycosylated human Ocn is more stable in human plasma in vitro compared to deglycosylated human Ocn.

Conclusion and relevance: Ocn O-glycosylation is a novel PTM which increases the stability of mouse Ocn in vitro and in vivo. Even though this modification does not naturally occur in human Ocn, the introduction of a single amino acid change in human Ocn is sufficient to induce its glycosylation in mammalian cells and to increases its stability in plasma. Thus, this simple modification provides a novel approach to improve human Ocn stability in future therapeutic applications for diabetes and obesity.
Environmental factors such as pollutants are increasingly being associated with the pathogenesis of diabetes. Of particular interest to the Bruin Lab is exposure to persistent organic pollutants (POPs) during critical stages of fetal development. Studies have shown that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a POP and potent inducer of cytochrome P450 (CYP) enzymes via the aryl hydrocarbon receptor, is correlated with an increase in metabolic disease following exposure. Further, data from our lab have indicated that POPs accumulate in the pancreas and induce CYP1A enzymes in both human and mouse pancreatic islets. We hypothesize that POPs may be accumulating in the pancreas, thereby eliciting stress on the developing beta cells through the aryl hydrocarbon receptor pathway. Using differentiation of human embryonic stem cells to mimic key stages of human pancreatic beta cell development in vitro, the goal of my research is to investigate how TCDD exposure might affect development and function of human beta cells. Constant, high-dose (10 nM) TCDD exposure during differentiation revealed changes in both morphology and expression of characteristic molecular markers as early as ‘Stage 2’ (primitive gut tube). Development toward the pancreatic lineage was significantly stunted under these conditions, as determined by flow cytometry analysis of key pancreatic markers (PDX1, NKX6.1). Current work is focusing on how lower TCDD doses may yield more subtle changes in beta cell differentiation and whether CYP enzymes are active during early human development. This research will contribute to existing data that point to environmental pollutants as sources of beta cell damage and dysfunction, thereby predisposing individuals to develop metabolic syndrome later in life.
Aging is associated with impaired stress granule formation in kidney cells

Ossama Moujaber¹, Dana Abou Samhadaneh¹, Ursula Stochaj¹

¹Physiology, McGill University

Organ functions decline during aging, and the most profound changes occur in the kidney. Notably, indicators of kidney dysfunction are detected in over 50% of elderly subjects, including those with Type 2 diabetes. Injury to the proximal tubule caused by oxidative stress is a major contributor to common forms of progressive chronic kidney diseases, such as diabetic nephropathy, affecting up to 40% of Type 1 or Type 2 diabetic patients. The polarized epithelium of the proximal tubule is particularly vulnerable to stress. To date, the aging-associated changes in proximal tubule physiology are not well-defined. Using cellular models of aging, our research is directed towards a better understanding of the changes.

The proper response to stress is crucial for cell and organ survival. The formation of cytoplasmic stress granules (SGs) is a conserved reaction that helps eukaryotic cells to survive stress. Aging impairs the stress response, but little is known about the underlying mechanisms.

It is our goal to define how aging compromises the kidney’s ability to cope with stress. To this end, we developed two models of renal proximal tubule cell aging. They are based on the chemical or pharmacological induction of senescence. We demonstrated that both model systems display hallmarks of aging.

Using these models, we assessed SG formation and stress-induced signaling. We showed that aging impairs SG assembly. Moreover, our studies uncovered the underlying molecular mechanisms.

Taken together, our research provides a better understanding of the aging-dependent changes in kidney physiology. We identified new biomarkers that can score the stress response in kidney proximal tubule cells. Long-term, this information will help to develop new diagnostic and therapeutic tools to evaluate the complications of the diabetic nephropathy.
Augmented levels of angiotensin-II (Ang-II) are associated with the development of cardiovascular disorders such as atherosclerosis and hypertension. Exaggerated activation of Ang-II-induced signaling events and expression of genes linked to cell proliferation, hypertrophy and migration have been demonstrated to contribute to the development of these vascular pathologies. Ang-II induces the phosphorylation of class II histone deacetylases (HDACs), particularly HDAC4 and HDAC5. HDACs regulate gene transcription by their ability to modify the acetylation status of the lysine residues in histone and non-histone proteins. A heightened activation of HDACs, notably HDAC5, has been associated with vascular disorders such as atherosclerosis. We have shown earlier that Ang-II induces the expression of early growth response factor-1 (Egr-1), a zinc finger transcription factor which is upregulated in atherosclerotic lesions and in animal models of vascular injury. We have also reported that the PI3Kinase/AKT pathway plays a key role in inducing the expression of Egr-1 in vascular smooth muscle cells (VSMC). However, the role of the AKT pathway in HDAC5 phosphorylation and the contribution of HDAC5 in Egr-1 expression in VSMC remains unexplored. Here, we show that Ang-II induced the phosphorylation of HDAC5 in a time-dependent manner, and pharmacological blockade of the PI3K/AKT pathway by wortmannin or SC66 attenuated Ang-II-induced HDAC5 phosphorylation in A10 VSMC. In addition, Ang-II treatment of VSMC resulted in the translocation of HDAC5 from the nucleus to the cytosol, and the inhibition of AKT by SC66 almost completely attenuated the nuclear export of HDAC5 induced by Ang-II. In contrast, pharmacological blockade of the MAPK pathway by UO126 was ineffective in reducing Ang-II-induced HDAC5 phosphorylation. Furthermore, pharmacological inhibition of HDAC5 by MC1568 or siRNA-induced silencing of HDAC5 suppressed Ang-II-induced Egr-1 expression. In summary, our results suggest that Ang-II induces the phosphorylation and nuclear export of HDAC5 via the PI3K/AKT-dependent signaling pathway and that HDAC5 is an upstream modulator of Egr-1 expression in VSMC.
41 - Defining the cardiac DPP4 cleavage peptidome

Natasha Trzaskalski\textsuperscript{1,2}, Shen Zhang\textsuperscript{3}, Brett Larsen\textsuperscript{3}, Karen Colwill\textsuperscript{3}, Benjamin Rotstein\textsuperscript{2}, Anne-Claude Gingras\textsuperscript{3}, Erin Mulvihill\textsuperscript{1,2}

\textsuperscript{1}University of Ottawa, \textsuperscript{2}University of Ottawa Heart Institute, \textsuperscript{3}Lunenfeld Tanenbaum Research Institute

In Canada, people with type 2 diabetes mellitus (T2DM) develop, and die, from heart disease 10-15 years earlier than people without T2DM. Medication currently in use focuses solely on either T2DM or heart disease, however, very few therapies address both issues at the same time. This project aims to investigate the role of a well-known protein that is elevated in people with T2DM and its relationship to heart disease. Dipeptidyl peptidase-4 (DPP4) is a widely expressed serine protease preferentially cleaving the N-terminal amino acids upstream of a penultimate proline in over 40 identified substrate proteins. DPP4-mediated cleavage plays a critical role in determining the half-life of many proteins. However, only 10% of the putative substrates have been validated within in vivo systems. Despite this, two drug classes based on inhibiting DPP4 cleavage are currently in use in millions of patients for the treatment of T2DM. Therefore the development of a method for identifying DPP4 substrates in vivo is of significant interest.

Using Hydrophobic Tagging-Assisted N-termini Enrichment (HYTANE) coupled with liquid chromatography tandem-mass spectrometry (LC-MS/MS) we enriched and identified the low abundant DPP4-generated substrates in complex biological samples (plasma, intestine, heart).

Proteins were extracted and isotopically labeled at the N-termini. After tryptic digestion, the peptides with labeled N-termini were separated from all others using HYTANE, fractionated using high-pH and analyzed by LC-MS/MS. Enrichment in neo-N-termini improved by 92.69% with reproducibility of 77% between technical replicates. In 3 biological replicates (WT and Dpp4 -/- mice), we consistently detected characterized DPP4 substrates.

With this method, we analysed ventricular tissue from older, dysglycemic, obese WT or mice treated with a DPP4 inhibitor. Gene-Ontology pathway analysis identified “abnormalities of the cardiovascular system and myocardium” as significantly enriched, and 5 previously, uncharacterized substrates met our strict, statistical criteria pipeline. These studies are the first to define DPP4 cleavage in vivo in complex tissues and have the potential to significantly improve our understanding of DPP4 action.
42 - Hedgehog Interacting Protein Overexpression in Renal Proximal Tubules Accelerates Renal Dysfunction in Mice Fed with High Fat Diet

Henry Nchienzia¹, Shiao-Ying Chang², MIN-CHUN LIAO³, Xin-Ping Zhao², Isabelle Chenier⁴, John Chan², Shao-Ling Zhang²,⁵

¹CRCHUM, Université de Montréal, ²CRCHUM, ³CRCHUM, ⁴CRCHUM, ⁵CRCHUM

Introduction: Hedgehog interacting protein (Hhip), characterized as a putative antagonist of hedgehog signaling, has been shown to be highly expressed in kidneys of diabetic mouse models. This increased Hhip expression has been correlated with diabetic nephropathy. The present study aims to establish a functional role of over expressing Hhip in renal proximal tubular cells (RPTCs) in a murine model of diet-induced obesity.

Methods: In vivo, both wild type (WT) and Hhip-transgenic mice (Hhip-Tg) male mice were randomly fed normal diet (ND) and high fat diet (HFD) from the age of 6 weeks old until 20 weeks respectively. Physiological parameters, intraperitoneal glucose challenge test (IPGTT), intraperitoneal insulin tolerance test (IPITT), glomerular filtration rate (GFR) and renal morphology were performed accordingly.

Results: Mice in HFD groups showed significantly greater weight gain as compared to mice fed ND. IPGTT revealed that HFD mice also developed pronounced glucose intolerance with no apparent changes in insulin sensitivity, systolic blood pressure, glomerular filtration rate and glomerular volume. Conversely, immunohistochemistry analysis revealed that HFD induced renal lipid deposition, and that HFD fed Hhip-Tg mice had a significant increase in renal lipid deposition compared to WT mice. Furthermore, renal expression of fatty acid binding protein 4 (FABP4) was more pronounced in HFD fed Hhip-Tg mice when compared to WT mice, respectively.

Conclusion: Our data suggest that in a diet-induced obesity model, overexpression of Hhip in RPTCs regulates renal lipid accumulation via FABP4 leading to lipotoxicity, which might contribute to the development of fibrosis.
43 - High Fat Diet Leads to Alterations of Hepatic Lipid and Amyloid β Metabolism in the 3xTg-AD Mouse Model of Alzheimer’s Disease

Cristina R. Bosoi1,2,4, Milene Vandal3,4, Marine Tournissac3,4, Hortense Fanet3,4, Jessica Virgili3,4, Robert Lippman5, Jasmohan S. Bajaj6, Andre Marette1,2, Frederic Calon3,4

1Centre De Recherche De L’institut De Cardiologie Et Pneumologie De Québec, 2Faculté De Médecine, Université Laval, 3Faculté De Pharmacie, Université Laval, 4Axe Neurosciences, Centre De Recherche Du CHU-Q (Pavillon CHUL), 5Mcguire VA Medical Center, VA, E-U, 6Virginia Commonwealth University, VA, E-U

Background: Fatty liver as well as cognitive and memory alterations are both known complications of obesity and type II diabetes. Alzheimer’s disease (AD), the most common cause of dementia, is increasingly considered as a peripheral metabolic disease. The presence of type II diabetes and obesity in midlife are well-known risk factors for developing AD at an older age. The relationship between AD and the liver has not been investigated yet. However, several liver-derived circulating lipid species have been proposed as biomarkers of AD. Amyloid β (Aβ) is a peptide with an unclear physiological function, which forms the amyloid plaques found in brains of AD patients and which is released in the circulation. The liver is an important Aβ clearing organ, yet its role during the disease remains unknown. The 3xTg-AD mouse model reproduces both Aβ and tau pathologies characteristic to AD neuropathology, but also peripheral metabolic impairments such as glucose intolerance. Since the liver plays a major role both in maintaining glucose and lipid homeostasis as well as in Aβ clearance, our aim was to explore the hepatic contribution to the peripheral metabolic alterations present in 3xTg-AD mice.

Methods: 3xTg-AD mice and non-transgenic mice on the same background (NonTg) received a high fat (HFD) or a control diet (CD) for 9 months and were sacrificed at 15 months of age. We assessed hepatic histology by hematoxilin-eozin staining and lipid content with commercial kits following chloroform-methanol extraction. Liver enzymes involved in lipid metabolism, gluconeogenesis and Aβ clearance were measured by qPCR and Western blot.

Results: Liver weight, hepatic lipid content and steatosis scores were not changed between NonTg and 3xTg-AD mice. Interestingly, these parameters increased following HFD only in NonTg, but not in 3xTg-AD mice. HFD decreased the phosphorylation level of liver acetyl-CoA carboxylase as well as expression of fatty acid synthase in NonTg and even more strikingly in 3xTg-AD mice (obese 3xTg-AD: p<0.05 vs obese NonTg for both enzymes). Expression of sterol regulatory element-binding protein 1 increased only in obese 3xTg-AD (p<0.05 vs all other groups). PPARα expression was decreased in HFD-fed NonTg mice and remained similar between obese 3xTg-AD and non-obese controls (obese 3xTg-AD: p>0.05 vs obese NonTg; p>0.05 vs NonTg). Gluconeogenic enzymes Pck1 and G6pc were not changed. Circulating Aβ and its hepatic receptor LRP1 were not changed between 3xTg-AD groups. However, expression of hepatic neprilysin, the main enzyme involved in the clearance of Aβ, significantly decreased in obese 3xTg-AD mice compared to all other groups.

Conclusion: Our results indicate that hepatic lipid accumulation is prevented in obese 3xTg-AD mice due to increased hepatic fatty acid oxidation and decreased lipogenesis. Hepatic clearance of Aβ is impaired by obesity in 3x-Tg-AD mice. Our results highlight the importance of peripheral metabolism in the pathogenesis of AD. A better comprehension of the mechanisms relating AD and peripheral metabolism may uncover potential therapeutic targets.
Lack of Tubular Heterogeneous Nuclear Ribonucleoprotein F (hnRNP F) Attenuates Kidney Hypertrophy and Glomerular Hyperfiltration in Diabetic Akita mice

Kana N. Miyata1, Chao-Sheng Lo1, Shuiling Zhao1, Isabelle Chenier1, Janos G. Filep2, Julie R. Ingelfinger3, Shao-Ling Zhang1, John S. D. Chan1

1CRCHUM, Université de Montréal, Montreal, QC, Canada, 2Research Centre, Maisonneuve-Rosemont Hosp., Montreal, QC, Canada, 3Pediatric Nephrology Unit, Mass. Gen. Hosp., Boston, MA, United States

Background: Kidney hypertrophy and glomerular hyperfiltration are known to precede the development of proteinuria and reduced renal function in patients with diabetes. The amelioration of glomerular pressure has been proposed as an underlying mechanism of the improved renal outcome in recent clinical studies with sodium-glucose co-transporter-2 (SGLT2) inhibitors, a new category of oral anti-diabetic agents.

We previously reported that a deficiency of heterogeneous nuclear ribonucleoprotein F (hnRNP F) in renal tubules down-regulates SGLT2 in non-diabetic mice (ASN Abstract, 2018). Non-diabetic tubule-specific hnRNP F knockout (KO) mice did not demonstrate any significant difference in blood glucose, kidney weight/body weight (KW/BW) ratio, or glomerular filtration rate (GFR) compared to control mice.

Objective: To investigate the impact of hnRNP F deficiency in diabetic mice at hyperfiltration stage.

Methods: Tubule-specific hnRNP F KO mice were generated as described previously via cross-breeding of Pax8-Cre mice with floxed hnRNP F mice on a C57BL/6 background. Akita-hnRNP F KO mice were created by cross-breeding of female tubule-specific hnRNP F KO mice with male heterozygous Akita mice. Male adult (8 weeks of age) Akita-hnRNP F KO mice, Akita mice, and control wild-type (WT) littermates were studied (n=4 per group). Blood glucose was measured by glucometer up to 24 weeks of age. At 24 weeks, GFR was measured by inulin-FITC clearance in awake mice prior to euthanization, and kidneys were processed for histology. The results are expressed as the mean ± SEM.

Results: Akita-hnRNP F KO mice had consistently lower blood glucose levels than Akita mice (WT 9.18±0.24 vs Akita 34.0 vs Akita-hnRNP F KO 28.0±2.69 at 24 weeks; p<0.01). Akita-hnRNP F KO mice had lower KW/BW ratio than Akita mice (WT 0.85±0.06 vs Akita 2.10±0.21 vs Akita-hnRNP F KO 1.44 ±0.13; p<0.01). In addition, GFR/BW ratio tended to be lower in Akita-hnRNP F KO mice than Akita mice (WT 6.23±0.19 vs Akita 17.40±5.48 vs Akita-hnRNP F KO 10.45±1.32). By histology, no significant signs of renal injury were observed in either of the groups. However, kidney tissues showed attenuated glomerulomegaly and decreased number of SGLT2-positive tubules in Akita-hnRNP F KO mice compared to Akita mice.

Conclusions: Our data demonstrate that kidney hypertrophy was attenuated in Akita-hnRNP F KO mice, likely by down-regulated SGLT2 lowering blood glucose levels as well as activating tubuloglomerular feedback, which decreases single nephron GFR. SGLT2 inhibitors are garnering attention for their renoprotective effects in diabetics. Our data indicate that Akita-hnRNP F KO mice can act as a unique preclinical tool to study the physiological effects of SGLT2 down-regulation.
45 - Littératie alimentaire et le diabète de type 1

Alexandra Itzkovitz1, Vanessa Magio1, Amélie Roy-Fleming1,2, Anne-Sophie Brazeau1,2

1Université McGill, 2Institut de recherches cliniques de Montreal

Objectif: Évaluer le niveau actuel de littératie alimentaire des jeunes adultes canadiens âgés 18 à 29 ans avec le diabète de type 1 (DT1), afin de développer des interventions thérapeutiques et de prévention des complications associées au DT1.

Méthodes: Ceci est une étude transversale, utilisant un questionnaire en ligne avec le logiciel Survey Monkey®. Les répondants sont recrutés à travers les médias sociaux. Le sondage inclus des questions concernant le statut socioéconomique, les connaissances et les attitudes en lien avec la nutrition. Le Short food literacy questionnaire y est également inclus.

Résultats: Parmi les 136 répondants avec le DT1, l'âge moyen est 24±3 ans, 94% sont caucasiens, 43% sont Québécois et 33% ont terminé des études universitaires. La plupart a déclaré avoir un contrôle du diabète adéquat (58,8% ont rapporté avoir une HbA1c comprise entre 6 et 7,5%). Bien que la majorité (76%) préparent leurs repas, seulement 43% des répondants ont rapporté avoir un niveau de confiance modéré quant à la capacité à préparer des repas savoureux et équilibrés. De plus, 56,6% rapportent ne pas suivre la méthode de l’assiette équilibrée et seulement 38% disent avoir confiance pour trouver de nouvelles recettes. Leurs principales sources d'informations nutritionnelles sont Internet (67,6%), suivis des étiquettes nutritionnelles (66,9%). Le score moyen en littératie alimentaire était de 39/52, ce qui correspond à un niveau de littératie alimentaire entre «problématique» et «suffisante».

Conclusion: Selon ces résultats préliminaires, le niveau de littératie alimentaire de cette population clinique est possiblement sous-optimal. Cette population ayant un risque de comorbidités cardiométaboliques, des interventions nutritionnelles sont requises.
46 - NRF2 Overexpression In The Renal Proximal Tubules Up-Regulates BMF Expression And Promotes BMF Induced Apoptosis.

Anindya Ghosh¹, Shuiling Zhao¹, Chao-Sheng Lo¹, Isabelle Chenier¹, Janos Filep², Julie R. Ingelfinger³, Shao-Ling Zhang¹, John SD Chan¹

¹CRCHUM, Université de Montréal, ²Maisonneuve-Rosemont Hospital, Research Centre, ³Harvard Medical School, Pediatr Nephrol Unit, Mass Gen Hospital, Boston, MA, USA

Background: Previously, we reported that Bmf (a pro-apoptotic protein) expression is differentially up-regulated in renal proximal tubular cells (RPTCs) in diabetic mice (Diabetes 2012). The underlying mechanism of up-regulation of Bmf expression in diabetes, however, is not well defined.

Objective: We investigated whether nuclear factor erythroid 2-related factor 2 (Nrf2) could regulate Bmf expression both in vivo and in vitro, and promote RPTCs apoptosis.

Methods: Transgenic mice overexpressing hBMF and NRF2 in the RPTCs were generated under KAP2 promoter. Systolic blood pressure, albumin creatinine ratio (ACR) were measured in male (12 to 20 weeks old) non-Tg, hBMF-Tg and NRF2-Tg mice. Protein and mRNA gene expression in isolated renal proximal tubules (RPTs) were assessed by Western blotting (WB) and quantitative polymerase chain reaction (qPCR). Apoptosis was measured by TUNEL assay. Rat immortalized PTCs (IRPTCs) with or without stably transfected with Bmf gene promoter were studied in vitro.

Results: hBMF-Tg and NRF2-Tg mice develop mild systemic hypertension and albuminuria, as well as increased Bmf expression and apoptosis in RPTCs compared to non-Tg mice. In vitro, Oltipraz (an Nrf2 activator) stimulated Bmf mRNA and gene promoter activity and reversed by trigonelline (an Nrf2 inhibitor) or siRNA against Nrf2. We further identified Nrf2-response element(s) in the Bmf gene promoter using promoter truncated experiment.

Conclusions: We conclude that chronic Nrf2 activation could induce RPTC apoptosis in diabetes, at least in part, via stimulation of Bmf gene transcription. These results firstly identify Nrf2 and Bmf as novel targets for the prevention of tubular apoptosis and atrophy in diabetes (Supported by CIHR).
47 - The development of an animal diet more representative of human consumption reveals a major role of dietary proteins in the development of obesity and type 2 diabetes

Choi B. S.-Y.1,2, Daniel N.1,2, Houde V.P.1,2, Vors C.1, Varin T.1, St-Pierre P.1,2, Marette A.1,2

1Institute of Nutraceuticals and Functional Foods (INAF), Laval University, 2Quebec Heart and Lung Institute (IUCPQ), Laval University

The study of obesity and cardiometabolic diseases via the use of animal models fed a diet high in fat and sucrose is very common. While much attention is paid to the sources of fat and carbohydrates used, very little attention is paid to proteins. Indeed, casein is often the only source of protein found in animal diets while it is known for its protective effect against the development of obesity compared to other sources of protein.

The objective is to study the impact of a protein mixture inspired by human consumption on metabolic health and gut microbiota.

We have developed a mixture of 10 protein sources respecting the proportions consumed by the American population (NHANES data) and included the mixture into a low fat and sucrose diet (LFLS) and a high fat and sucrose diet (HFHS). C57BL/6J mice were fed with these diets or control diets containing only casein as a source of protein for 12 weeks. Feces were collected at several time points to analyse gut microbiota, an oral glucose tolerance test (OGTT) was performed at week 11 and tissues such as muscle, liver, adipose tissues were collected at sacrifice for further analysis.

The use of a protein mixture amplifies the effects of the HFHS diet on the development of obesity, glucose intolerance and hyperinsulinemia compared to animals consuming only casein, whereas no difference were observed on these parameters between the two LFLS groups. For the gut microbiota composition, we found significant modulations of several bacteria, such as Akkermansia muciniphila that was enhanced in mice eating casein compared to mice eating the protein mixture, in both HFHS and LFLS. Diversity was also modulated by the protein source and was lower in the mice fed the casein diets. These changes were also represented in gut microbiota functions, as we observed changes of SCFA and BSCFA profiles related to the diet type and the protein source. Preliminary determination of insulin signaling in the liver revealed that the protein mixture resulted in greater activation of the mTORC1/S6K1 pathway and inhibitory phosphorylation of IRS-1 on Ser1101, leading to greater inhibition of Akt activation by insulin.

This study demonstrates the importance of considering a diverse source of dietary proteins consumed by humans when using animal models to represent diet-induced obesity and type 2 diabetes in humans. Our results reveal that when compared to casein alone, this mixture of proteins changes gut microbiota, potentiates the effect of the obesogenic diet and induces a more pronounced insulin resistance and impaired glucose tolerance phenotype that is more representative of human pathology.
INTRODUCTION: Nonalcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver biochemistry in North America. It is now estimated that 10-22% of hepatocellular carcinoma (HCC) cases are attributed to fatty liver disease. However, the mechanisms underlying the relationship between diet-related hepatic metabolic disease and cancer development are poorly understood. PGC-1α is a master regulator of metabolism. Interestingly, low levels of PGC-1α are reported in patients diagnosed with inflammatory fatty liver disease and human HCC. We aim to determine whether low hepatic PGC-1α, when combined with a western diet, potentiates the development of liver cancer.

METHODS: We developed a mouse model of NAFLD-associated liver cancer by combining a high-fat/high-fructose diet (HFHFD) with a "second hit" of diethylnitrosamine (DEN). Female and male mice expressing either one (LH, liver heterozygotes) or two (LKO, liver knockouts) floxed PGC-1α alleles under the control of the albumin promoter were subjected to the protocol. Weights were monitored weekly and serum samples taken bi-monthly. Body composition, liver tumour multiplicity and maximum size were quantified. RNA and protein samples from adjacent liver tissues and tumours were used to investigate pathways known to influence nutrient metabolism, oxidative stress and hepatocarcinogenesis.

RESULTS: We observed a decrease in PGC-1α expression in liver tumours of mice given the carcinogen (DEN) in combination with the high-fat/high-fructose diet. We also observed a similar trend in human HCC. After quantifying liver tumour multiplicity and maximum size in whole livers of mice subjected to NAFLD-induced HCC protocol, our data shows that LKO male mice exhibit increased liver tumour number and maximum size. Interestingly, loss of hepatic PGC-1α significantly reduces oxidative metabolism, G1-phase marker yH2AX and apoptotic marker cleaved-caspase 3. This reduction is associated with a significant increase in p-ERK and p-STAT3 in adjacent liver tissues. Also, tumours from LKO male mice exhibit upregulated B-catenin/TCF target genes (Cyclin D1, MYC, CD44, SOX9). Finally, gain- and loss-of-function experiments show that PGC-1α regulates E-cadherin gene expression and B-catenin subcellular localization.

In conclusion, loss of hepatic PGC-1α combined with an obesogenic diet promotes NAFLD-associated liver cancer. Moreover, loss of hepatic PGC-1α may impact the G1 checkpoint entry, cell survival and cancer stem cell potential, in part, through differential B-catenin subcellular localization. Our data implicates PGC-1α as an important mitigating factor in the development of diet- and NAFLD-associated liver cancer.