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1 Distinct Role of mTORC1 and S6K1 in the Regulation of Hepatic Glucose Metabolism.

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The Ser/Thr kinase mTOR (mammalian Target of Rapamycin), which is the catalytic core of the multi-protein complex mTOR complex 1 (mTORC1) and its downstream mediator S6K1 (p70 S6 kinase) are well-known regulators of metabolism. Moreover, inhibition of mTORC1 and/or S6K1 may be an interesting therapeutic target for combating insulin resistance. We have recently confirmed that chronic inhibition of mTORC1/S6K1 by a selective inhibitor, rapamycin, improves insulin signaling by increasing IRS-1/PI3K activity *in vivo*. Unexpectedly, despite enhanced or maintained IRS/PI3K signaling in these models, chronic down regulation of mTORC1/S6K1 also paradoxically causes insulin resistance and glucose intolerance due to major perturbations in transcriptional pathways regulating hepatic gluconeogenesis. However, is mTORC1, or S6K1, or both molecules critical for the regulation of gluconeogenesis? Here, we show that chronic rapamycin treatment increased IRS-2 activity and binding to PI3K but impaired Akt translocation to the cell membrane and increased hepatic glucose production in hepatocytes. These results are in agreement with our previous *in vivo* studies. Conversely, selective inhibition of S6K1 by PF-4708671 (PF, 10 μ M) improved insulin signaling. Moreover, chronic inhibition of S6K1 reduced hepatic glucose production in hepatocytes in the basal state and potentiated insulin effects. We also observed a significant effect of S6K1 inhibition on phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) gene expression, two important enzymes involved in gluconeogenesis. Indeed, hepatocytes treated with PF showed a strong decrease in PEPCK and G6Pase gene expression after only 1 hour of treatment. These results contrast with those obtained with chronic rapamycin treatment and suggest that mTORC1 and S6K1 are distinct regulators of hepatic glucose metabolism. In conclusion, S6K1 inhibition could represent an interesting therapeutic target to improve hepatic insulin action.

2 The Protective Effect of a Novel Salmon Peptide Fraction on Metabolic Syndrome in a Mouse Model of Obesity and Atherosclerosis

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Our lab has shown that the effects of isolated proteins from different fish species ameliorate the metabolic profile of high fat, high sucrose (HFHS)-fed obese rats (1). Fish proteins were found to reduce inflammatory mediators in visceral fat and the salmon protein hydrolysate (SPH) also influenced fat mass and insulin sensitivity. In addition to the work on fish proteins, we have shown that fish oil feeding prevents fat accretion, reduces fasting glycemia and normalizes glucose tolerance and insulin sensitivity in HFHS-fed rats (2). Consequently, we wanted to identify the most promising bioactive peptide from fish protein hydrolysate. Our *in-vitro* experiments have shown that a salmon peptide hydrolysate (SPH) of fractionated peptides under 1 kDa improved insulin-stimulated glucose uptake in myocytes, decreased glucose production of hepatocytes and attenuated macrophage inflammatory profile (unpublished results). The objective of this project was to test this SPH in combination or not with omega-3 fatty acids in LDLr^{-/-}/ApoB^{100/100}, a mouse model prone to develop obesity and atherosclerosis, and evaluate its effects on metabolic functions and at the genomic level. Our preliminary results revealed that the mice treated with the SPH, despite having high-fat high sucrose diet induced obesity, showed improved glucose tolerance, increased insulin and C-peptide secretion and reduced hepatic glucose production which was only partially potentiated by the addition of omega-3 fatty acids in the early phase of the treatment. These results suggest an excellent potential of the SPH and will help us to conduct a clinical study to evaluate if this SPH improves some features of the metabolic syndrome in obese, insulin-resistant humans as we have found in mice. Our long-term goal is to develop a new socially acceptable and commercially viable functional food or nutraceutical containing these bioactive peptides.

1. Pilon G, Ruzzin J, Rioux LE, et al. Differential effects of various fish proteins in altering body weight, adiposity, inflammatory status, and insulin sensitivity in high-fat-fed rats. *Metabolism*. 2011;60:1122-1130.

2. Samane S, Christon R, Dombrowski L, et al. Fish oil and argan oil intake differently modulate insulin resistance and glucose intolerance in a rat model of dietary-induced obesity. *Metabolism*. 2009;58:909-919.

3 Non-Catalytic Region of Tyrosine Kinase Adaptor Protein 2 (Nck2) is not a Major Player in Regulating Insulin Sensitivity in Adipose Tissue

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Many individuals suffer from Type 2 Diabetes, a disorder which leads in part to an inability to respond to insulin; a phenomenon known as insulin resistance. Non-catalytic region of tyrosine kinase adaptor proteins (Nck), Nck1 and Nck2, are known to play a role in intracellular signal transduction from activated receptor tyrosine kinases. A role for Nck1 in mediating insulin resistance has previously been established: Nck1 knockout (KO) mice are protected against insulin resistance. Conversely, Nck2 KO mice display insulin resistance suggesting that Nck2 is required for normal insulin sensitivity. However, the insulin-sensitive tissue in which this resistance is manifested remains to be elucidated. This study aimed to determine whether adipose tissue is a contributor to the insulin resistance seen in Nck2 KO mice. Experiments described herein demonstrate that adipose tissue of wild-type (WT) and Nck2 KO mice showed no difference in the phosphorylation levels of two Akt residues, markers of insulin signaling. In addition, no difference in Nck1 expression was observed between adipose tissue of WT and Nck2 KO mice. These findings suggest that depletion of Nck2 does not affect insulin signaling in adipose tissue. Furthermore, given that Nck1 levels did not change in the absence of Nck2, Nck1 is not the major player responsible for retaining normal insulin signaling in adipose tissue, but rather this is an Nck2-dependent effect. Other insulin sensitive tissues are currently under evaluation to identify which is responsible for insulin resistance in Nck2 KO mice. Determining the exact mechanism behind insulin resistance will be useful in understanding several diseases that are characterized by insulin resistance, including type 2 diabetes.

4 Relationship between Duration of Lactation and Metabolic and Anthropometric Profiles Among Women with Prior Gestational Diabetes

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Lactation has been associated with a reduced long-term risk of type 2 diabetes and obesity for the mother, but few data are available in women with prior gestational diabetes mellitus (GDM).

AIM: To investigate the relationship between total duration of lactation and metabolic and anthropometric profiles in women with prior GDM.

METHODS: The study group included 177 women with a history of GDM between 2003 and 2010 for whom metabolic and anthropometric profiles were available. Mean duration between last GDM-complicated pregnancy and metabolic testing was 4.0±1.9 years. Waist circumference, weight and height were measured and body mass index (BMI) was calculated. Body fat percentage was measured using bioelectrical impedance. Glycemia and insulin values were obtained from a 75g oral glucose tolerance test (OGTT) and were used to calculate the HOMA-IS and the MATSUDA indexes for estimating insulin sensitivity. Metabolic syndrome was defined according to the NCEP ATP III criteria. Lifetime duration of lactation was self-reported by the participants. Results were adjusted for age, parity, energy intake (kcal), the use of insulin during pregnancy and current physical activity practice.

RESULTS: Mean age was 36.3±4.9 years and mean BMI was 27.3±6.2 kg/m². Duration of lactation was positively associated with HOMA-IS and MATSUDA indexes for insulin sensitivity ($r=0.16$, $p=0.04$ and $r=0.18$, $p=0.02$, respectively) and negatively correlated with fasting plasma insulin ($r=-0.17$, $p=0.02$), plasma triglycerides concentrations ($r=-0.18$, $p=0.02$) and body fat percentage ($r=-0.18$, $p=0.03$). Women who breastfed ≥9 months (stratified according to the median) had higher HOMA-IS and MATSUDA indexes as well as reduced fasting plasma insulin, 2h post OGTT plasma insulin and plasma triglycerides concentrations compared to women who breastfed < 9 months ($p<0.05$ for all). Women who breastfed < 9 months were also at increased risk for the metabolic syndrome (OR: 2.47, IC 95% [1.01-6.06]).

CONCLUSIONS: These results suggest that longer duration of lactation is associated with improved metabolic and anthropometric profiles as well as a reduced risk of metabolic syndrome among women with prior GDM.

5 Impact of Cod Protein on Insulin Sensitivity and Plasma Lipids in Women with Polycystic Ovary Syndrome: Preliminary Data

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OBJECTIVE: The objective of our study was to compare the effects of cod protein to those of other animal proteins on glucose tolerance, insulin sensitivity and plasma lipids in women with polycystic ovary syndrome (PCOS).

METHODS: As preliminary data, two women with PCOS aged between 18 and 45 years old were fed a cod protein semi-controlled diet and a similar diet containing beef, pork, veal, eggs, milk and milk products (BPVEM) for 3 months each, within a crossover design study. Prior to and after each experimental period, glucose tolerance and glucose disposal rate (GDR) were determined by oral glucose tolerance test (OGTT) and hyperinsulinemic-euglycemic clamp, respectively. The concentrations of cholesterol and triglycerides were measured in plasma, LDL and HDL.

RESULTS: After 3 months of intervention, the CP diet increased GDR by 32%, whereas the BPVEM diet reduced it by 21%. Also, the CP diet reduced triglycerides and cholesterol to HDL ratio by 37% and 15% respectively, while the BPVEM diet decreased these parameters by 17% and 10%. HDL cholesterol increased by 12% with the CP diet and by 3% with the BPVEM diet.

CONCLUSIONS: These preliminary results suggest that CP diet can increase glucose disposal rate and HDL cholesterol and lower triglycerides in women with PCOS, improving their cardiometabolic risk profile. The study is ongoing and these data need to be confirmed with a greater cohort of subjects. Supported by CDA (Operating grant) and Diabète Québec (Studentship).

6 Short-term Changes in Dietary Intakes, Anthropometric Variables, Metabolic Profile and Quality of Life Following a Nutritional Education Program Based on the Self-Determination Theory

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BACKGROUND: Health professionals are facing an important challenge when trying to motivate individuals to make dietary changes in order to improve their health.

OBJECTIVES: To determine differences between men and women in adherence to the Mediterranean diet and in changes in anthropometric variables, metabolic profile, eating behaviors and quality of life in response to a 12-week nutritional education program promoting the Mediterranean diet.

METHODS: The 12-week intervention is based on the Self-Determination Theory and uses a Motivational Interviewing approach. It includes 3 group sessions and 7 individual sessions with a dietitian. A Mediterranean score was calculated with a validated food frequency questionnaire. Motivation was evaluated with the Regulation of Eating Behavior Scale. Anthropometric and metabolic variables were measured with standard methods. Eating behaviors and quality of life were evaluated with the Three-Factor Eating questionnaire and the SF-36 questionnaire, respectively. The Mixed procedure was used to evaluate gender and time effects, as well as gender by time interactions. Preliminary results were analyzed with a group composed of 25 men and 20 women (aged between 20 and 50 years old).

RESULTS: The Mediterranean score increased in response to the 12-week nutritional intervention in men and women (time effect, $p=0.0001$), but without significant gender differences. Men decreased significantly more their motivation level in response to the intervention compared to women (interaction gender x time, $p=0.003$). Women increased significantly more their flexible restraint level (interaction gender x time, $p=0.02$) and decreased more their internal hunger level (interaction gender x time, $p=0.047$) in response to the intervention, compared to men. Quality of life improved (time effect, $p=0.02$) in both men and women in response to the nutritional education program. Whereas women significantly decreased more than men their waist to hip ratio (interaction gender x time, $p=0.0046$), both men and women showed decreases in triglyceride concentrations (time effect, $p=0.0009$), increases in HDL-C concentrations (time effect, $p<0.0001$), and decreases in total-C/HDL-C ratio (time effect, $p<0.0001$) in response to the intervention.

CONCLUSION: Preliminary results suggest that the nutritional education program based on the Self-Determination Theory, using a Motivational Interviewing approach, allowed changes in dietary intakes, beneficial changes in the anthropometric and metabolic profiles, and improvement in quality of life in men and women.

7 Associations of Sex Hormone-binding Globulin Concentrations with Anthropometric and Metabolic Variables in Men and Women

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BACKGROUND: Some studies have suggested that a high sex hormone-binding globulin (SHBG) concentration is associated with a more favorable metabolic profile in men and women. This association could be partly explained by the inverse correlation between SHBG and adiposity.

OBJECTIVE: To verify how SHBG concentrations are correlated to anthropometric and metabolic variables in men and women showing a slightly deteriorated metabolic profile.

DESIGN: A total of 37 men and 32 premenopausal women took part to this study. Participants were between 25 and 50 years of age and were characterized by a slightly elevated plasma LDL-cholesterol (C) concentration (between 3.4 and 4.9 mmol/L) or total cholesterol to HDL-C ratio ≥ 5.0 . Measurements were performed after a 12-h fast. Insulin and glucose levels were measured following a 75g oral glucose load. Data reported here correspond to measurements performed at baseline of a 4-week nutritional intervention based on the Mediterranean diet.

RESULTS: Analyses were adjusted for body mass index (BMI). In men, SHBG concentrations were inversely associated with TG ($r=-0.33$, $p=0.049$), fasting insulin ($r=-0.33$, $p=0.049$) and 2-h insulin concentrations ($r=-0.36$, $p=0.03$) and directly associated with HDL-C ($r=0.36$, $p=0.03$) and adiponectin concentrations ($r=0.47$, $p=0.004$). In women, SHBG concentrations were inversely associated with waist circumference ($r=-0.54$, $p=0.003$) and fasting insulin concentrations ($r=-0.39$, $p=0.04$) and directly associated with HDL-C ($r=0.40$, $p=0.03$) and apo A-1 concentrations ($r=0.43$, $p=0.02$) as well as with insulin sensitivity as determined by the HOMA index ($r=0.38$, $p=0.045$).

CONCLUSION: Our results suggest that a high SHBG concentration is associated with a more favorable anthropometric and metabolic profile in men and women even after adjustment for BMI.

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8 Relationship between the Adoption of a Healthy Diet and the Metabolic Profile in Women with Prior Gestational Diabetes

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WOMEN with prior gestational diabetes mellitus (GDM) are at increased risk for type 2 diabetes. The adoption of a healthy diet is encouraged in order to prevent or delay this risk.

OBJECTIVE: To evaluate the relationship between the adoption of a healthy diet and the metabolic profile in women with prior GDM.

METHODS: The analysis included 154 women who had GDM between 2003 and 2010. Diet quality was evaluated from the information obtained with a validated food frequency questionnaire. A diet quality score, derived from the *Alternate Healthy Eating Index (AHEI)*, was obtained for each subject. Women were then classified as having adopted a healthy diet (highest tertile of the maximum possible score derived from the AHEI) or not. Weight and height were measured and body mass index (BMI) was calculated. Fasting insulinemia and glycemia were obtained and the HOMA index for insulin sensitivity (HOMA-IS) was calculated. Analyses were adjusted for age, parity and energy intake.

RESULTS: Participating women were 36.0 ± 4.8 years old and mean BMI was 27.3 ± 6.4 kg/m². 44.8% of women did not adopt a healthy diet as previously described. The diet quality score was positively correlated with the HOMA-IS index ($r=0.24$, $p=0.003$) and negatively correlated with fasting insulinemia ($r=-0.23$, $p=0.005$) and BMI ($r=-0.17$, $p=0.04$). Compared to women who adopted a healthy diet, women who did not adopt a healthy diet were more likely to have a BMI ≥ 25 kg/m² (OR: 2.28, 95% CI [1.10-4.75]), to have fasting insulinemia ≥ 74.5 pmol/l (OR: 2.15, 95% CI [1.05-4.41]) and to have a lower insulin sensitivity (HOMA-IS index < 0.053 , OR 2.08, 95% CI [1.02-4.26]). No association was observed with fasting glycemia ($p>0.05$).

CONCLUSION: These results suggest that women with prior GDM who do not adopt a healthy diet in the years following delivery are more likely to be overweight or obese and to have lower insulin sensitivity.

9 Nutritional and Hormonal Signals Modulate Fatty Acid Oxidation in the Hypothalamus

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The hypothalamus plays a critical role in the control of food intake and peripheral metabolism. This control relies in part on the detection of circulating levels of nutrients such as glucose (Glu) and fatty acids (FAs) by the mediobasal hypothalamus (MBH). Recent evidence suggests that the ability of FAs to modulate energy homeostasis relies on its intracellular metabolism. Specifically, oxidation of FAs may be a key step involved in the effects of FAs and hormonal signals such as ghrelin to regulate food intake. The objective of the present work was to directly measure hypothalamic FAs oxidation, determine whether it is modulated by nutritional and hormonal signals and identify some of the potential mechanisms involved. We measured FAs oxidation in hypothalamic neuronal cell lines and in MBH explants from male Wistar rats using palmitate (Pal) radioactive tracers. Our results show that Glu utilization and oxidation increase while Pal oxidation decreases in response to increased Glu concentrations (1, 4, 8 and 15 mM) in GT1-7 and N-46 neuronal cell lines, an effect that was mimicked by etomoxir, an inhibitor of carnitine palmitoyltransferase-1 (CPT-1)-mediated mitochondrial FAs transport. Increasing glucose concentrations reduced AMP-activated protein Kinase (AMPK) phosphorylation in N-46 neurons and treatment with Compound C, an inhibitor of AMPK, decreased Pal oxidation in the presence of low glucose. In ex vivo experiments, our data show that Pal oxidation in the MBH is significantly elevated compared to cortical controls and is decreased in response to etomoxir. Moreover, rats fed a high-fat diet for 3 days had increased Pal oxidation in the MBH. Finally, treating MBH explants with ghrelin (100 or 300 nM) increased phosphorylation of AMPK and Pal oxidation. Our results suggest the existence of a metabolic coupling between Glu and FAs involving the AMPK/Malonyl-CoA/CPT-1 network. In addition, ghrelin treatment and high-fat diet, which increase food intake, enhance FAs oxidation rate in the MBH. Collectively, these data support the notion that FAs oxidation in the hypothalamus constitutes a signal involved in the control of food intake.

10 Gastric Leptin: Secretion by the Epithelial Cells, Transport to the Duodenal Lumen, Absorption by the Enterocytes, Transport to Circulation and Putative Physiologic Importance in Energy Metabolism

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Leptin first described as an adipokine, is a hormone playing important roles on the nutritional and energy status of the organism as well as on the regulation of food intake. It is secreted in an endocrine way by the adipose tissue and in an exocrine way by the gastric mucosa. Gastric epithelial chief cells of the lower half of the gastric mucosa express and secrete leptin. Quantitative immunogold has revealed that leptin is processed along the conventional RER-Golgi-granule secretory pathway of the epithelial cells together with other classical gastric enzymes such as pepsinogen and lipase. Upon food intake, the content of the secretory granules is released into the gastric lumen. Leptin resists the drastic proteolytic conditions of the gastric juice due to its binding to a protective protein present in the secretory granules. Characterization of this binding protein revealed that it corresponds to the soluble isoform of the leptin receptor. Once secreted, the complex leptin-leptin binding protein crosses the pyloric sphincter and reaches the duodenum. Membrane bound leptin receptors lining the intestinal cell microvilli interact with the luminal leptin. Among other actions, the binding of leptin to this receptor regulates absorption of nutrients and intestinal mucosa integrity. Interestingly, some of the luminal leptin is endocytosed by the enterocytes through clathrin-coated vesicles and upon crossing the Golgi is transcytosed to the baso-lateral space to be delivered to the blood circulation, reaching its hypothalamic target cells and regulating satiety and food intake. In vitro knock-down experiments, using human intestinal Caco-2 cell line revealed that this transcytosis is an active process dependent on the long isoform of the leptin receptor. Thus, gastric leptin plays important paracrine and endocrine actions for the control of gastric emptying, intestinal nutrient absorption and for the regulation of food intake by the central nervous system.

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