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Obesity in humans can lead to metabolic problems such as glucose intolerance and insulin resistance, which may result from pancreatic islet dysregulation and reduced insulin sensitivity in the liver. LEW.1WR1 (1WR1) rats became more glucose intolerant than LEW/SsNHsd (SsNHsd) rats after 12 weeks on a moderate sucrose diet.1 We hypothesize that the 1WR1 rats develop decreased insulin sensitivity due to impaired islet function and liver responses to insulin. To test this hypothesis we measured blood hormone levels and islet and liver gene expression. The terminal blood insulin (14988+/- 4024 vs. 22703+/-5101 pg/mL; p=0.0085; n=7,7) and glucagon (127.3+/-73.31 vs. 188.6+/-46.87 pg/mL; p=0.0537; n=7,7) were higher in the the 1WR1 rats. Using qRT-PCR, we determined the islets of 1WR1 rats had 3 fold increased insulin (p<0.0001; n=3,3) and glucagon (p<0.0001; n=3,2) relative gene expression. Yet, the β-cell area (22.05+/-6.408 vs. 2.276 +/-1.284mm2; p=0.0016; n=3,4) was significantly reduced in 1WR1 rats. Islet Plin5 expression was upregulated in 1WR1 rats (5.388+/-0.3806 F.C.; p<0.0001; n=3,3) indicating increased lipid droplet production, while Cyclin D (0.5726+/-0.08797 F.C.; p=0.0035; n=3,2) was downregulated indicating decreased cell cycle proliferation. These results indicate that the islets of the 1WR1 rats were insensitive to insulin signaling, which may have been caused by increased lipid droplets and a decrease in compensatory islet area. We also measured the relative expression of insulin-sensitive genes in the liver tissue to determine if there were alterations in liver insulin signaling. Downregulation of Irs-2 (0.5840+/-0.001045 F.C.; p<0.0001; n=7,7) expression was likely caused by the upregulated fat10 gene in 1WR1 rats.2 Fat10 (2315+/-0.01380 F.C.; p<0.0001; n=4,6) expression in the liver was significantly increased. Foxo1 (2.644+/- 0.001211 F.C.; p<0.0001; n=7,7) expression, which is normally reduced by insulin, was upregulated which indicates reduced insulin sensitivity. Upregulated expression of Fgf21 (2.260+/-0.002376 F.C.; p<0.0001; n=6,7), which improves glucose homeostasis, in the liver is why the fasting blood glucose of 1WR1 rats were not significantly different from the SsNHsd rats.1 In conclusion, 1WR1 rats show increasingly impaired metabolism over time. These rats have increased insulin and glucagon levels coupled with liver fat10 overexpression leading to impaired gene regulation of insulin-responsive genes in the liver. These changes synergistically increase susceptibility to pathological obesity and metabolic disease.

References: (1) Collins et al., Journal of the Endocrine Society. 2019 3(S1). (2) Ge, Q. et al., Frontiers in Physiology. 2018; 9(1051): 1–16.

Diabetes Mellitus and Glucose Metabolism
IMPACTS OF ORGAN CROSSTALK AND SEX ON DIABETES PHENOTYPES

Growth Hormone Receptor Gene Disruption in Mature-Adult Mice Improves Glucose Metabolism and Lifespan in Females

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Growth hormone (GH) serves an important role in early and adult life. Reduction of GH action has been shown to increase life span in many species of animals. In fact, mice bearing a congenital disruption of GH receptor (GHR) gene (GHRKO) hold the record for the longest-lived laboratory mice. In addition to extended life span, these mice show improved health with lower rates of cancer, increased insulin sensitivity, and resistance to age-associated cognitive decline. Furthermore, humans with decreased GH action due to inactivating mutations in the GHR (Laron Syndrome patients) are resistant to cancer and diabetes. Even though the beneficial effects of congenital *Ghr* gene disruption are well studied, the consequences of postnatal disruption of GH action were unknown. Previously our laboratory generated a mouse line with disrupted GH action at 1.5 months of age (1.5mGHRKO mice). Results showed that these mice had improved insulin sensitivity and increased maximal lifespan only in females, yet growth retardation was still present.

To consider decreased GH action as a possible therapeutic to extend healthy lifespan, it was imperative to elucidate the effects of disrupting *Ghr* gene at a mature-adult age, well after the developmental and growth period of the mice. To this end, we hypothesized that removal of GH action in adult life would convey some of the same health and life span benefits seen in the GHRKO mice without the reduced body length. To test this hypothesis, we used the cre-lox system to generate mice with a disrupted *Ghr* gene at a mature-adult age (6 months), referred as 6mGHRKO mice. We then performed a phenotypic and lifespan characterization, and tested for molecular mechanisms known to be associated with extended longevity, namely oxidative stress resistance and mTOR modulation. We found that similar to GHRKO and 1.5mGHRKO mice, disruption of GHR at 6 months of age resulted in mice with increased adipose tissue mass, decreased lean mass, high circulating GH, but decreased insulin growth factor-1 levels compared to control mice. Furthermore, the 6mGHRKO mice displayed significantly improved insulin sensitivity in males, with no changes in glucose tolerance. Also, serum levels of inflammatory markers and liver triglycerides were unchanged in these mice. Experiments to evaluate the status of oxidative damage and mTOR activation in liver, skeletal muscle and subcutaneous adipose tissue of male and female 6mGHRKO mice showed a tissue-specificity and sexual dimorphism in these results. Importantly, male and female 6mGHRKO mice showed no change in body length, but mean, median and maximal lifespan were significantly extended in females. In conclusion, disruption

of GH action well past sexual maturation produces beneficial effects on insulin sensitivity and aging in mice.

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High-Fat Diet Accelerates Pathological Progression and Intestinal Inflammation in a Type 2 Diabetes Rodent Model

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Insulin signaling lowers postprandial glucose by stimulating cell surface translocation of the insulin sensitive glucose transporter 4 (GLUT4). In order to better understand how insulin resistance contributes to the pathophysiological progression of type 2 diabetes, we generated human *GLUT4* promoter-driven insulin receptor knockout (GIRKO) mice and characterized their metabolic features relative to control mice. Although the role of insulin resistance in diabetes is beyond dispute, our previous studies showed that GIRKO mice fed normal chow diet (NCD) had an unexpectedly low rate of frank diabetes despite severe insulin resistance in muscle, fat, and brain.

In the current study, we first sought to determine whether GIRKO mice would respond to high-fat diet (HFD) challenge with worsened glycemic outcome compared to control mice on HFD. Secondly, we sought to determine whether HFD-induced pathologies in GIRKO mice were caused by adaptations in the gastrointestinal (GI) tract and microbiome. We discovered that after beginning the HFD-feeding regimen, GIRKO mice rapidly developed hyperinsulinemia and hyperglycemia without excessive adiposity gain. Furthermore, GIRKO mice displayed dyslipidemia via increased hepatic lipid accumulation and serum lipid content. We used indirect calorimetry to characterize the metabolic features of single-housed mice. HFD-fed GIRKO mice had comparatively lower respiratory exchange ratio (RER), indicating relatively greater lipid metabolism compared to control mice on HFD. Despite having increased circulating incretins, GIRKO mice had impaired oral glucose tolerance and limited glucose-lowering benefit from Exendin-4 (Ex-4) injections. Since HFD promotes inflammation in the gastrointestinal (GI) tract, we performed gene expression analysis and pathway analysis of duodenal mRNAs to investigate whether inflammatory response, glucose transport, and lipid transport were altered in HFD-fed GIRKO mice. Among the top pathways discovered in pathway analysis were those involved with inflammatory signaling, carbohydrate transport, and xenobiotic metabolism, which supports that HFD-fed GIRKO mice have increased GI tract inflammation which may promote impaired glucose homeostasis.

In conclusion, our studies suggest that HFD increased intestinal inflammation and exacerbated insulin resistance, which catalyzed the pathological progression of diabetes. Future studies are necessary to identify the molecular and cellular signaling pathways which culminate in frank diabetes, which may lead to therapeutic targets for regulating glucose homeostasis in the context of insulin resistance.

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Insulin Resistance and Gender Define a Cell Autonomous Supernetwork of Protein Phosphorylation

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Many hormones and growth factors, including insulin, act through networks of protein phosphorylation. Insulin resistance is an important factor in the pathophysiology of many metabolic disorders. The aim of this study was to uncover the cell autonomous determinants of insulin action and protein phosphorylation using induced pluripotent stem cell (iPSC)-derived myoblasts (iMyos) in vitro. Here, we show that iMyos from non-diabetic individuals in the highest quintile of insulin resistance show impaired insulin signaling, defective insulin-stimulated glucose uptake and decreased glycogen synthase activity compared to iMyos from the insulin sensitive individuals, indicating these cells mirror in vitro the alterations seen in vivo. Global phosphoproteomic analysis uncovered a large network of proteins whose phosphorylation was altered in association with insulin resistance, most outside the canonical insulin-signaling cascade. More surprisingly, we also observed striking differences in the phosphoproteomic signature of iMyos derived from male versus female subjects, involving multiple pathways regulating diverse cellular functions, including DNA and RNA processing, GTPase signaling, and SUMOylation/ubiquitination. These findings provide new insights into the cell autonomous mechanisms underlying insulin resistance in the non-diabetic population and provide evidence of a major, previously unrecognized, supernetwork of cell signaling differences in males and females that must be considered in understanding the molecular basis of sex-based differences in normal physiology and disease.

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Liver-Specific Expression of Constitutively Active $G_s\alpha$ Leads to Hyperglycemia With Impaired Insulin Secretion