site significantly decreased the promoter activity in mouse hepatoma cells (Hepa1-6) and mouse primary hepatocytes, and the promoter carrying the mutated HNF-1 site was not transactivated by co-transfected HNF-1 in a non-hepatic cell line. These findings indicated that HNF-1 was essential and critical factor for the basal expression of Angptl8 in murine liver. In fact, knockdown of Hnf-1 using siRNA method in mouse Hepa1-6 and mouse primary hepatocytes reduced Angptl8 protein levels. We also performed Electrophoretic mobility-shift assays and confirmed the direct binding of Hnf-1 to its *Angptl8* promoter binding motif. To elucidate whether refeeding could enhance HNF-1, we checked the expression levels of Hnf-1 in mouse liver. Hnf-1 expression levels of both mRNA and protein were increased after short-term refeeding, paralleling the enhanced expression of the Angptl8. Moreover, insulin-stimulated primary hepatocytes showed increased expression of Angptl8 protein, but knockdown of Hnf-1 completely abolished this enhancement by insulin. Chromatin immunoprecipitation (ChIP) analyses confirmed the recruitment of endogenous Hnf-1 to the *Angptl8* promoter region and it was strongly induced by insulin. Conclusion: HNF-1 plays essential role in hepatocyte-specific and refeeding-induced rapid increases in *Angptl8* expression via insulin.

Adipose Tissue, Appetite, and Obesity

NOVEL MECHANISMS CONTROLLING ADIPOSE TISSUE PHYSIOLOGY AND ENERGY BALANCE

Hepatic GH Receptor Signaling Directly Suppresses Hepatic Steatosis and** De Novo **Lipogenesis, Independent of Changes in Plasma IGF1 and Insulin

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A reduction in GH, as well as IGF1, is associated with non-alcoholic fatty liver disease (NAFLD). However, the relative contribution of changes in circulating GH and IGF1, to hepatic triglyceride accumulation (steatosis), remains to be clearly defined. To study the direct actions of GH on hepatocyte metabolism, we have utilized a mouse model of adult-onset, hepatocyte-specific, GHR knockdown (aHepGHRkd; 10–12 week-old, $GHR^{fl/fl}$ male mice, treated with AAV8-TBGp-Cre). In this and previous reports, we have observed that aHepGHRkd male mice rapidly develop steatosis (after 7 days) associated with enhanced *de novo* lipogenesis (DNL; measured by deuterated H_2O labeling, 10h after 0800h food removal), and low ketone levels, suggestive of reduced hepatic β-oxidation. Of note, aHepGHRkd also reduces plasma IGF1 levels to >80% of GHR-intact controls ($GHR^{fl/fl}$ mice treated with AAV8-TBGp-Null), leading to a rise in GH, due to loss of IGF1 negative feedback to the pituitary/hypothalamus. This reciprocal shift in IGF1/GH is associated with an increase in insulin levels. Therefore, it is possible that the steatosis that develops in aHepGHRkd mice is the consequence of systemic insulin resistance supplying excess substrates (glucose and NEFA) for hepatic lipogenesis. However, inconsistent with this theory is the fact that glucose and NEFA levels are not altered after aHepGHRkd. To tease out the indirect (perhaps driven by high insulin levels) vs. direct effects of GH on hepatocyte lipid accumulation, male aHepGHRkd mice were injected with a vector expressing rat IGF1 (AAV8-TBGp-rIGF1). Reconstitution of hepatocyte IGF1 in aHepGHRkd mice, raised plasma IGF1 and normalized GH, insulin and ketone levels, but hepatic steatosis and DNL remained greater than that of GHR-intact controls, indicating GH directly suppresses hepatic fat accumulation. RNAseq analysis of livers from aHepGHRkd mice showed expression of genes related to carbohydrate metabolism (*Gck*, *Khk*) and fatty acid synthesis (*Fasn*, *Srebf1, Usf1*), processing (*Scd1*) and uptake (*Cd36*) were increased, while genes related to gluconeogenesis (*Pck1, Fbp1, G6pc*) were reduced. Remarkably, IGF1 reconstitution had no major impact on the hepatic transcriptome of aHepGHRkd mice, with the exception of reducing the expression of *Srebf1*, consistent with the reduction in circulating insulin levels. Interestingly, carbohydrate-responsive element-binding protein (CHREBP) levels, but not mRNA levels, were greater in aHepGHRkd mice with or without IGF1 reconstitution, consistent with upregulation of CHREBP target genes (*Khk* and *Fasn* among others). Taken together, these results suggest GH directly regulates steatosis, at least in part, by suppressing carbohydrate-driven DNL, where additional studies are underway to test this hypothesis.

Adipose Tissue, Appetite, and Obesity

NOVEL MECHANISMS CONTROLLING ADIPOSE TISSUE PHYSIOLOGY AND ENERGY BALANCE

HepatocyteGHR/STAT5b Signaling Protects Against Liver Injury in NAFLD/NASH Mice Models Independent of Steatosis

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Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of pathologies ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) that can lead to cirrhosis and hepatocellular carcinoma. Clinical and mouse studies indicate GH-signaling is reduced in NAFLD. We reported that chow-fed mice, with adult-onset, hepatocyte-specific GH receptor knockdown (aHepGHRkd) develop steatosis, and with age, a mild NASH-like phenotype. In the present study, we sought to determine if aHepGHRkd accelerates the development of steatosis and fibrosis in the context of diets shown in wild-type male mice, after 6 months of feeding, to produce mild NASH (60% fat [lard] + sucrose in the drinking water [HFS] or a severe NASH-like phenotype (40% fat,

with partially hydrogenated corn-oil; 2% cholesterol; 20% fructose [HFCF]). Since aHepGHRkd is associated with a reduction in active STAT5b, aHepGHRkd mice were treated with either a hepatocyte-specific adenoviral-associated vector that expresses constitutively active STAT5b (AAV-TBGp-STAT5b^{CA} = STAT5b^{CA}) or a AAV-Null vector. After only 3 months of feeding either the HFS or HFCF diet, aHepGHRkd, but not GHR-intact controls, mice exhibited clear fibrosis, associated with higher levels of plasma alanine aminotransferase (ALT). STAT5b^{CA} treatment of aHepGHRkd mice reduced fibrosis, as well as plasma ALT. Of note, hepatic TG content did not differ between the treatment groups, within diet. Preliminary studies used GC-MS to reveal aHepGHRkd, in the context of HFS diet, increased hepatic fatty acid ratios indicative of enhanced *de novo* lipogenesis, while STAT5b^{CA} reversed this effect. These results suggest GHR/STAT5b may protect against liver injury not by controlling absolute fat accumulation, but by modifying the fatty acid composition of hepatic lipids. Finally, in order to determine if STAT5b^{CA} could also reverse established diet-induced NASH, wild-type mice were fed the HFCF diet for 6 months and then treated with AAV-STAT5b^{CA} or AAV-Null vectors, and followed for an additional 3 months. Preliminary findings show STAT5b^{CA} modestly reduced liver weight with no changes in TG content. However, STAT5b^{CA} prevented the rise in plasma ALT observed in Null-treated controls. Of note, some mice developed hepatic tumors, where the number and size of visible tumors was reduced by STAT5b^{CA}. Importantly, in all models examined thus far, changes in the liver phenotype could not be clearly attributed to changes in systemic metabolism, supporting a direct action of GHR/STAT5b signaling on liver health. Taken together, these results suggest that enhancing hepatocyte STAT5b activity could prevent/treat diet-induced NASH. How STAT5b mediates these effects, and if there are other players involved, remains to be elucidated.

Adipose Tissue, Appetite, and Obesity

NOVEL MECHANISMS CONTROLLING ADIPOSE TISSUE PHYSIOLOGY AND ENERGY BALANCE

Hypothalamic Glucagon Receptors Regulate Feeding in Mice

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Glucagon is an essential regulator of glucose and lipid metabolism. We have reported that chronic glucagon receptor (GCGR) activation with the highly selective, long-acting GCGR-agonist, IUB288, promotes weight-loss by stimulating energy expenditure and suppressing food intake in diet-induced obese (DIO) mice. Thus, novel therapeutics that include glucagon receptor (GCGR) agonism have emerged as promising candidates for obesity and diabetes. GCGR-stimulated energy expenditure is predominately dependent on hepatic GCGR activation; however, the tissue(s) responsible for GCGR-dependent suppression of food intake have yet to be elucidated. Intriguingly, intracerebroventricularly (ICV) injected glucagon acutely suppresses food intake, suggesting neurons expressing GCGR in the brain mediate the anorectic actions of GCGR activation. Hypothalamic neurons express appetitive neuropeptides, sense nutrients in circulation, and respond to peripheral endocrine signals. Studies herein, utilize mice with hypothalamic *Gcgr*-deficiency (*Gcgr*$^{\Delta Hypo}$) to test the hypothesis that peripherally administered GCGR-agonists (e.g. IUB288) reverse obesity via their actions on hypothalamic GCGRs to suppress food intake and concurrent hepatic effects on energy expenditure. *Gcgr*$^{\Delta Hypo}$ and littermate control mice were fasted overnight to stimulate endogenous hunger signals and test for differential food intake upon refeeding. Interestingly, lean, male *Gcgr*$^{\Delta Hypo}$ mice displayed acute hyperphagia in comparison to control littermates. *Gcgr*$^{\Delta Hypo}$ mice also displayed elevated locomotor activity, an increase in the respiratory exchange ratio, and elevated energy expenditure compared to littermate controls. Furthermore, these metabolic alterations are associated with delayed body weight gain and chronic hyperphagia in *Gcgr*$^{\Delta Hypo}$ mice allowed ad libitum access to a high fat diet for 12 weeks. Consistent with our hypothesis, chronic peripheral administration of IUB288 (14d i.p.) suppressed food intake in DIO male control, but not *Gcgr*$^{\Delta Hypo}$, mice. Altogether, these data suggest that hypothalamic GCGRs mediate the anorectic actions of GCGR activation and play a regulatory role in food take. Moreover, these findings suggest that GCGR-based therapeutics may act on both intake and expenditure components of energy balance to combat obesity.

Adipose Tissue, Appetite, and Obesity

NOVEL MECHANISMS CONTROLLING ADIPOSE TISSUE PHYSIOLOGY AND ENERGY BALANCE

Identification of Key Genes and Pathways for Childhood Obesity Using System Biology Approach Based on Comprehensive Gene Information

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Introduction: Childhood obesity is one of the most important public health issues of the 21st century. Epidemiological studies have suggested that obesity during childhood increases the risk of developing comorbidities, such as type 2 diabetes, later in life. Childhood obesity is a complex disease whose molecular mechanisms are not completely elucidated. In this context, a system biology approach could contribute to the scientific knowledge regarding genetic factors related to childhood obesity onset. **Aim:** To identify molecular mechanisms involved in childhood obesity by implementing a system biology approach. **Methods:** Experimentally validated and computationally predicted genes related to Pediatric Obesity (C2362324) were downloaded from the DisGeNET v7.0 database. The protein-protein interaction (PPI) network was constructed using the STRING v11.0 database and analyzed using NetworkAnalyst v3.0 and Cytoscape v3.8.1. The relevance of each node for the network structure and functionality