The liver
Key in regulating appetite and body weight

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Liver fructose-1,6-bisphosphatase (FBPase) is a regulatory enzyme in gluconeogenesis that is elevated by obesity and dietary fat intake. Whether FBPase functions only in glucose metabolism or has other metabolic roles is currently unclear. In our recently published study, we examined the importance of liver FBPase in body weight regulation by performing a series of comprehensive physiological and biochemical assessments of energy balance and specific intervention studies in our transgenic mouse line that overexpresses FBPase specifically in the liver. Compared with negative littermates, these FBPase transgenic mice weighed 10% less, had 50% less adiposity, ate 15% less food but did not have altered energy expenditure. Increased circulating leptin and cholecystokinin levels, elevated fatty acid oxidation and reduced appetite stimulating neuropeptides, neuropeptide Y (NPY) and agouti-related peptide (AGRP), underpinned this phenotype. Blocking the action of FBPase returned food intake and body weight to those of the negative littermates. Our study is the first to identify liver FBPase as a previously unknown regulator of appetite and adiposity. Importantly, this work recognizes the liver as an important organ in appetite and body weight regulation. This commentary will provide further insight and expand on this novel concept that the liver does in fact play an important role in adiposity.

Liver as an Important Appetite Regulating Organ

Classical regulation of appetite occurs via a fine network of signals from various peripheral tissues that integrate within the hypothalamus. The ability to sense and respond to changes in the animal’s nutritional status makes the hypothalamus a critical tissue in appetite and body weight regulation. This central control mechanism primarily involves activation of a number of centrally mediated signals produced by either “first order” or “second order” neurons. The “first order” neurons are the fundamental responders and are located within the arcuate nucleus (ARC) of the hypothalamus. They express the orexigenic neuropeptides, agouti-related peptide (AgRP) and neuropeptide Y (NPY) and the anorexigenic neuropeptides, cocaine and amphetamine regulated transcript (CART) and pro-opiomelanocortin (POMC).1 The actions of these neuropeptides occur via projections to “second order” neurons expressing eating behavior-modifying neuropeptides such as corticotrophin releasing hormone, orexins and melanin concentrating hormone.

Obesity has reached epidemic proportions throughout the world not only as a disease on its own but as a contributor to many other disease processes, such as type 2 diabetes, cardiovascular disease and obstructive sleep apnea. Obesity is an imbalance between energy consumed and energy expended leading to increased energy storage. While we understand the basic components involved in body weight regulation, the overall mechanism is not entirely understood. This is undoubtedly an essential pre-requisite if we are to identify novel targets and develop more effective therapies to combat this disease.
Communication peripherally arises from acute satiation signals released from the gastrointestinal tract (GIT) and secreted during meals that are combined with more tonically active adiposity signals that adjust energy balance accordingly. These satiation signals reflect the nutrient and caloric content of a meal and include the hormones cholecystokinin (CCK), peptide YY, glucagon-like-peptide 1, oxyntomodulin and ghrelin (stimulator of food) from the GIT and pancreatic polypeptide and amylin from the pancreas. The most well documented adiposity signal is leptin, which is secreted in direct proportion to adipose tissue mass and has longer lasting effects on adiposity stores compared with the satiation signals. Insulin too is an important long-term regulator of adiposity, being released by the pancreatic β-cell in proportion to fat mass. Together, both these hormones are accurate indicators of stored energy. It is this synergistic relationship between peripheral signals that is critical in relaying accurate information on whole body nutritional status to the hypothalamus to adjust energy intake and expenditure accordingly.

While most of the hormones secreted from the GIT (stomach and intestines) are known to be involved in the regulation of appetite and body weight, there is a body of evidence suggesting that the liver is important in energy homeostasis. Studies by Rossetti and colleagues have demonstrated that the brain can communicate directly to the liver to regulate glucose production through hypothalamic nutrient sensing to both glucose and fatty acids and vagal nerve actions. Our study has now shown the reverse with a liver-brain link that controls appetite and the amount of fat stored as adipose tissue. Early work in the 1960s indeed suggested the liver may have been a regulator of feeding behavior, but this was largely ignored and further work to define its role was required. In the past two decades studies were performed that suggested a potential link between liver metabolism and control of food intake. Specifically, infusion of the fructose analog 2,5-anhydro-D-mannitol into rodents increased food intake that was reversed following selective vagotom or capsaicin treatment of the vagus nerve. Similarly, increased food intake was observed following liver-specific fatty acid oxidation (FAO) inhibition using the fatty acid inhibitor, 2-mercaptoacetate. It was proposed that a reduction in liver ATP levels was the cause, but how the liver communicated to the brain was not determined. Interestingly, increasing liver FAO had the opposite effect with reduced food intake but again no insight into causative mechanisms. Therefore while there was precedence in the literature for a role of the liver in appetite regulation, the data was indirect with little understanding of how it occurred.

Role of Liver FBPase in Body Weight Regulation

Consuming excess fat results in excess weight gain primarily through an increase in total calories consumed. Interestingly, when rodents are fed high-fat (HF) diets, they eat less in terms of grams of food per day. This generally occurs in the early phase of HF exposure with animals increasing their intake following longer-term consumption. This implies that the animals try to compensate for the excess calorie load by eating less food and suggests the existence of a mechanism that is sensitive to excess nutrient intake and is activated to protect the animal from gaining too much weight. HF diets are also well documented to cause differential expression of a multitude of genes in various tissues. FBPase is one such gene in which our laboratory has been interested for several decades. We have shown FBPase to be elevated in the livers of HF-fed animals and in polygenic animal models of obesity such as the New Zealand Obese (NZO) mouse. Other laboratories have shown similar upregulation in islet β-cells incubated with HF, in other obese animal models (ob/ob mouse and fatty fatty rat) and in obese humans with type 2 diabetes.

In its primary role, FBPase is one of three key regulatory enzymes involved in the production of endogenous glucose from the liver and to a lesser extent, the kidney, with PEPCK and G6Pase making up the triad. Its main action is to convert fructose-1,6-bisphosphate (F1,6P) into fructose-6-phosphate (F6P) and opposes the glycolytic reaction catalyzed by phosphofructo-1-kinase. Given the intimate role of FBPase in glucose production, we naturally presumed that overexpression of such an important enzyme would lead to increased glucose production, glucose intolerance and perhaps type 2 diabetes. Our laboratory therefore proceeded to produce two transgenic mouse lines that overexpressed human FBPase under the control of the transthyretin promoter. This led to an increase in FBPase of 2- to 3-fold at the mRNA, protein and activity levels, but no discernible differences in glucose tolerance. An increase in the conversion of glycerol into glucose was the only observation of increased glucose production. To our surprise, both these transgenic lines weighed 10% less than their negative littermates, leading us to propose that liver FBPase may have a novel role in body weight control.

To investigate this potential regulatory role of FBPase, we performed a series of comprehensive physiological and biochemical assessments in hemizygous liver-specific FBPase transgenic mice and their negative littermates. The lean body weight phenotype was maintained in the transgenic mice throughout adulthood and was consistent with our previous publications. Most intriguing was the observation that these mice had a > 50% reduction in white adipose tissue mass. Reduced food intake rather than increased energy expenditure was associated with this leanness. To search for the underlying cause, we measured anorexigenic and orexigenic neuropeptide levels within the ARC of our mice and found a clear reduction in both NPY and AgRP mRNA expression levels but no change in POMC levels. One could argue that we may have produced mice with hypothalamic FBPase expression, which could have accounted for the neupeptide and body weight effect. While we did find low expression in the hypothalamus of our first line of transgenic mice, the second line used in this study contained insulator sequences to confer tissue specificity. This insulined line of FBPase transgenic mice expressed the same level of FBPase transgene as the original mice, but was specific to the liver with no ectopic expression in the hypothalamus or other tissues. Importantly, the transgenic mice still exhibited the same...
lean body weight phenotype. Furthermore, our laboratory also generated transgenic mice expressing the human FBPase gene specifically in the islet β-cell and showed no effect on body weight. Together our data provide solid evidence that liver FBPase plays an important role in the regulation of appetite and adiposity.

To confirm our findings, we proceeded with various intervention studies in which we directly targeted liver FBPase or the primary communication link between liver and brain. As FBPase was seen as a novel target by the pharmaceutical industry for type 2 diabetes control, we were able to use a commercially available pharmacological inhibitor of the human FBPase gene [benzoxazole benzene sulfomide (Calbi-ochem)] specifically to target our construct. Following ten days of inhibitor treatment, our transgenic mice normalized their food intake to the level of the controls and a parallel increase in body weight and NPY/AgRP mRNA expression levels. Strikingly, the transgenic mice regained their adipose tissue mass to that of the controls, even after ten days of treatment. These results implied that liver FBPase was in fact an important enzyme in controlling appetite and adiposity. The vagus nerve is the primary link between liver and brain, possessing both stimulatory and inhibitory effects on food intake. By performing hepatic branch vagotomy that disengages this link in our transgenic mice, food intake and consequently body weight was increased compared with control mice, demonstrating a requirement for vagal nerve signaling in our model. In fact, when we measured circulating leptin and CCK levels in our mice (two of the main hormones acting on this nerve to regulate food intake) we found both to be ~30% higher in the transgenic mice than controls. By blocking the vagal nerve, CCK1 receptor using a specific pharmacological antagonist (lorglumide), we were able to reverse food intake in the transgenic mouse to that of the control. However, our most impressive finding was the data from the polygenic obese and insulin-resistant mouse model, the NZO mouse. This mouse model is known for its increased endogenous glucose production and glyceral conversion to glucose from the liver. Early studies by our laboratory showed increased liver FBPase protein and activity levels in the NZO mouse. Treatment with the same FBPase inhibitor for seven days demonstrated a sustained increase in food intake and body weight compared with vehicle treated NZO mice. While we were surprised to see this result, we were also reassured by a similar increase in body weight in another model of obesity and diabetes, the ZDF rat. This result along with our intervention studies supports and validates our hypothesis that upregulating liver FBPase may not have a role in disturbing glucose metabolism per se, but instead play a significant role in the homeostatic mechanism to control body weight in states of nutrient excess.

Our finding of increased plasma leptin in the presence of reduced adipose tissue mass was intriguing. Leptin is said to be positively correlated with adiposity so while our finding was interesting in terms of the increase in plasma levels, it is not uncommon as there have been reports of leptin levels not showing a direct correlation with fat mass in some rodent models. While the importance of leptin at the physiological level is well described, what regulates adipose tissue leptin at the transcriptional level is limited. Endogenously, leptin secretion is controlled by factors such as insulin, glucose, glucocorticoids (that increase secretion) and cytokines, free fatty acids and leptin itself (that decrease secretion). At the transcription level only a few factors have been identified including C/EBPα, SREBP1 and most recently FOSL2. It may be possible that upregulation of FBPase activates a specific signal(s) from the liver to the adipose tissue to stimulate leptin production and secretion. We do know that adipose tissue communicates with other tissues through adipokine secretion (e.g., leptin) and there is some evidence in vitro that hepatocytes and adipocytes crosstalk leading to enhanced metabolic regulation, but whether the opposite is true is unclear.

Dissecting the Pathway Linking the Liver to Appetite and Adiposity Regulation

Having established a defined link between the liver and the brain to control appetite and adiposity, the question remained; what was the causative mechanism? Our group previously published a positive association between elevated CCK levels and the byproduct of fatty acid oxidation (FAO), the ketone body 3-β-hydroxybutyrate (BHB) in humans. Since we observed an increase in circulating CCK in our transgenic mice we followed this line of thinking and looked at the relationship between liver FBPase and FAO. We found elevated FAO at both a whole body level (through indirect calorimetry) and in the liver through increases in both the rate-determining enzyme carnitine palmitoyl-CoA transferase (CPT-1a) and circulating BHB levels. Treating transgenic mice with an acute dose of etomoxir (a specific inhibitor of CPT-1a) reversed the effects and led to an increase in food intake. We therefore proposed that increasing FAO was the main mechanism underpinning this phenotype.

As with the role of the liver in appetite regulation, the role of BHB in reducing food intake was touched upon in the early 1970s and 1980s and not investigated further. Only recently has this view re-emerged as an important research area. The evidence is now building not only from our FBPase transgenic mouse but also directly from a cohort of wild-type mice following an acute injection of BHB. We showed lowered food intake 2 h into the dark cycle and elevated circulating CCK levels in these treated mice and is supported by another study suggesting that FAO-induced reduction of food intake in rats was CCK mediated, since blockade of the CCK1 receptor significantly increased food intake. Very little is known about the cellular mechanism mediating the anorectic effect of BHB but our work has now established that CCK may be an important factor in this system and warrants further investigation.

The Hexosamine Biosynthesis Pathway: Linking Liver FAO to Reduced Appetite and Adiposity

In seeking the reason for the increase in FAO we considered whether alteration in ATP levels and induction of the AMPK pathway was the primary link. Under
normal circumstances, reducing ATP levels would increase AMPK to generate more ATP through pathways such as FAO. Lowered ATP occurs when glycolysis is suppressed (as would be expected when gluconeogenesis is increased) and as we saw in our islet β-cell specific FBPase transgenic mice. However, when we measured both ATP and phospho-AMPK levels, neither was upregulated in our transgenic mice suggesting an AMPK-independent pathway. As stated earlier, FBPase catalyzes the reaction converting the substrate (F1,6P) to the product (F6P). Both metabolites were increased in our transgenic mice and are likely related to the elevated glycerol gluconeogenesis and circulating glycerol levels in the mice. F6P is the main entry substrate for the hexosamine biosynthesis pathway (HBP), a pathway proposed as a cellular sensor of energy availability in muscle and fat. The main enzyme of this pathway, O-linked N-acetylglucosamine transferase (OGT), has been documented to form a complex with the FAO regulator, PGC-1α. This led us to investigate this pathway more closely. We found that hepatic OGT was elevated at both the mRNA and protein levels in our transgenic mice favoring increased flux through the HBP. Additionally, PGC-1α and another FAO regulator, PPARα, were also upregulated in the transgenic liver. We therefore proposed that upregulating liver FBPase increased flux through the HBP to increase OGT and activate FAO via PGC-1α, PPARα and CPT-1a. The byproduct BHB, as a result, was elevated leading to reduced food intake via elevated CCK and leptin. We do acknowledge that while our data demonstrates a plausible mechanism of action, it is complex. There is a possibility that FBPase may activate other pathways given that there are a number of important parameters involved. For example, liver FBPase could directly stimulate or interact with other liver derived proteins/metabolites that could signal afferent pathways to elicit both peripheral and central responses. Moreover, FBPase could directly stimulate FAO to induce the downstream responses. Even if other pathways are involved, the important message from our work is that the liver directly communicates with the brain to control appetite and body weight.

**Conclusions and Future Perspectives**

Our study provides a comprehensive examination of how an upregulation of a specific liver gluconeogenic enzyme can have a profound and independent effect on appetite and adiposity regulation without altering glucose metabolism. Elevating liver FBPase leads to increased FAO, overproduction of BHB, stimulation of CCK and leptin release and the generation of a vagal signal leading to reduction in the anorexigenic neuropeptides, NPY and AgRP to reduce food intake. We propose that under normal circumstances, nature provided us with an innate system to deal with excess fat in the diet by increasing liver FBPase to act as a negative feedback mechanism and reduce any further weight gain that would occur when too much fat was consumed. Clearly, in our current environment of constant nutrient excess this biochemical system is over-ridden and promotes increased weight gain.

The data also highlights the deleterious effects of weight gain and increased adiposity if FBPase is targeted and suppressed for better glycemic control. Pharmaceutical companies currently view FBPase as an attractive therapeutic target for type 2 diabetes due to its direct inhibition of glucose production and glycogenolysis compared with available treatments that act primarily on insulin resistance or insulin deficiency. However, unlike the insulin-sensitizing thiazolidinediones, which increase subcutaneous fat mass but decrease visceral fat mass, the use of FBPase specific inhibitors will increase fat mass across all depots thereby potentially worsening metabolic control in those with type 2 diabetes.

While our study has certainly highlighted the importance of the liver as an organ in the control of appetite and adiposity, it has also provided us with some very interesting and thought provoking questions that warrant further research: (1) How does BHB and ketosis in general orchestrate control over appetite? (2) Why is there an increase in plasma leptin when adiposity is reduced? and (3) Could our transgenic animal model also provide a potential liver-fat link that along with our liver-brain link reinforces the role of the liver in body weight regulation? We now have the opportunity to answer some of these questions when this model is studied in greater detail and better understand the intricacies behind appetite, adiposity and overall body weight regulation.

**Acknowledgments**

We would like to acknowledge Professor Joseph Proietto [Department of Medicine (Austin Health), The University of Melbourne, Heidelberg, VIC Australia] for providing critical review and opinion into this commentary. We would also like to acknowledge the National Health and Medical Research Council (NHMRC) of Australia for providing the funding to complete this work (APP566784).
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