Brief Communication

MOLECULAR METABOLISM

A critical role for ChREBP-mediated FGF21 secretion in hepatic fructose metabolism

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ABSTRACT

Objective: Increased fructose consumption is a contributor to the burgeoning epidemic of non-alcoholic fatty liver disease (NAFLD). Recent evidence indicates that the metabolic hormone FGF21 is regulated by fructose consumption in humans and rodents and may play a functional role in this nutritional context. Here, we sought to define the mechanism by which fructose ingestion regulates FGF21 and determine whether FGF21 contributes to an adaptive metabolic response to fructose consumption.

Methods: We tested the role of the transcription factor carbohydrate responsive-element binding protein (ChREBP) in fructose-mediated regulation of FGF21 using ChREBP knockout mice. Using FGF21 knockout mice, we investigated whether FGF21 has a metabolic function in the context of fructose consumption. Additionally, we tested whether a ChREBP-FGF21 interaction is likely conserved in human subjects.

Results: Hepatic expression of *ChREBP-β* and *Fgf21* acutely increased 2-fold and 3-fold, respectively, following fructose gavage, and this was accompanied by increased circulating FGF21. The acute increase in circulating FGF21 following fructose gavage was absent in ChREBP knockout mice. Induction of *ChREBP-β* and its glycolytic, fructolytic, and lipogenic gene targets were attenuated in FGF21 knockout mice fed high-fructose diets, and this was accompanied by a 50% reduction in de novo lipogenesis a, 30% reduction VLDL secretion, and a 25% reduction in liver fat compared to fructose-fed controls. In human subjects, serum FGF21 correlates with de novo lipogenic rates measured by stable isotopic tracers ($R = 0.55$, $P = 0.04$) consistent with conservation of a ChREBP-FGF21 interaction. After 8 weeks of high-fructose diet, livers from FGF21 knockout mice demonstrate atrophy and fibrosis accompanied by molecular markers of inflammation and stellate cell activation; whereas, this did not occur in controls.

Conclusions: In summary, ChREBP and FGF21 constitute a signaling axis likely conserved in humans that mediates an essential adaptive response to fructose ingestion that may participate in the pathogenesis of NAFLD and liver fibrosis.

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Keywords FGF21; ChREBP; Fructose; Lipogenesis; NAFLD

1. INTRODUCTION

Increased sugar consumption is a contributor to the worldwide epidemic of obesity and its associated complications [1]. The increasing prevalence of obesity is paralleled by a less obvious epidemic, that of NAFLD [2]. While hepatic steatosis, the first stage in NAFLD, is considered relatively benign with respect to liver disease per se, it may progress to non-alcoholic steatohepatitis (NASH) and may progress further to cirrhosis or hepatocellular carcinoma. Sugar consumption, particularly in the form of sugar-sweetened beverages (SSBs), is associated with the development and progression of NAFLD independently of other features of obesity and the metabolic syndrome [3,4].

Sugar is typically consumed by humans in the form of sucrose or high fructose corn syrup (HFCS), both of which consist of nearly equal amounts of the simple sugars, glucose and fructose. The fructose component of sugar appears to be particularly harmful as excessive consumption of fructose, but not glucose, increases visceral adiposity, serum triglycerides, and insulin resistance [5]. Fructose consumption also stimulates DNL more than glucose [5–7], which contributes to the development of steatosis and NAFLD [8,9]. The differential effects of glucose and fructose on NAFLD and other features of metabolic syndrome are, in part, due to the fact that fructose is preferentially metabolized in the liver (reviewed in [10,11]). We have recently demonstrated that fructose, but not glucose, ingestion acutely and robustly activates hepatic Carbohydrate Responsive-Element Binding Protein (ChREBP) a key carbohydrate sensing transcription factor that regulates glycolytic, fructolytic, and lipogenic gene expression programs [12]. ChREBP KO mice are intolerant to fructose and have

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reduced hepatic triglyceride levels and hepatic DNL rates on high-starch diets [13]. Thus, fructose may contribute to hepatic steatosis through at least two mechanisms: 1) by providing substrate for fatty-acid synthesis and esterification in the liver and 2) by stimulating hepatic lipogenic gene programs under the control of ChREBP and other signaling factors [reviewed in [14]].

Fibroblast growth factor 21 (FGF21) is a metabolic hormone synthesized by multiple tissues and released into circulation largely by the liver [15–17]. Pharmacological administration has multiple beneficial metabolic effects [18]. FGF21 was initially identified as a liver hormone regulated by fasting and ketogenic diets under the transcriptional control of PPAR-alpha, a master regulator of fatty acid oxidation [15,16,19,20]. The liver enriched transcription factor CREBH interacts with PPAR-alpha and also participates in regulating FGF21 expression after fasting or high-fat, low-carbohydrate feeding [21,22]. In mice, FGF21 participates in an adaptive response to fasting or ketogenic diets by enhancing hepatic fatty acid oxidation and ketogenesis [15,20]. In humans, however, neither short term fasting nor consumption of ketogenic diets markedly alter circulating FGF21 levels. In contrast, in humans, fructose ingestion leads to a robust and rapid increase in circulating levels of FGF21, which return to baseline after several hours. Thus FGF21's function in humans may be unrelated to fasting physiology [23–25].

Interestingly, in mice, high sucrose diets also increase FGF21 [26,27]. The sugar-sensitive transcription factor ChREBP can transactivate expression of hepatic *Fgf21* in vitro and in animal models [27], leading to the hypothesis that sucrose- or fructose-mediated activation of hepatic ChREBP may regulate circulating FGF21, which, in turn, might participate in an adaptive metabolic response to sugar ingestion. An adaptive role for FGF21 in sugar consumption is supported by human population genetics data indicating that variants in the human *FGF21* locus regulate carbohydrate consumption [28,29] as well as recent genetic and pharmacological interventions in rodents and primates indicating that FGF21 regulates sweet taste preferences [30,31].

Here, we show that the acute FGF21 response to fructose ingestion observed in humans is conserved in mice. Furthermore, using ChREBP KO mice and FGF21 KO mice, we demonstrate that ChREBP is essential for fructose-induced increases in circulating FGF21. Moreover, we show that FGF21 is required for a normal hepatic metabolic response to fructose consumption and that the absence of FGF21 leads to liver disease in mice on high-fructose diets. Lastly, we show that circulating FGF21 in humans correlates with rates of DNL indicating that a sugar-ChREBP-FGF21 signaling axis may play a role in the pathogenesis of NAFLD in humans.

2. MATERIALS AND METHODS

2.1. Animals

All studies were carried out using female mice obtained from and maintained at 24 °C on a 12:12-h light–dark cycle. ChREBP KO mice were obtained from The Jackson Laboratory (Stock No: 010537; Bar Harbor, ME) and back crossed more than 10 generations onto the C3H/HeJ background. FGF21-KO mice were generated as previously described [15] and back crossed more than 10 generations onto the C57BL/6J background. Mice were fed a chow diet (LabDiet 5008, Pharmaserv, Framingham, MA), a 60% fructose diet, or a 60% dextrose diet (TD.89247 and TD.05256 Harlan Teklad, Madison, WI) for indicated durations. Mice were euthanized between 9:00 and 11:30 am under isoflurane anesthesia. All studies were approved by the Beth Israel Deaconess Medical center IACUC.

2.2. Measurements in human subjects

Fourteen of fifteen healthy lean and overweight adult volunteers who participated in a previously published study [7] had given consent for the use of their stored plasma samples for future research. The measurement of levels of FGF21 in these samples was approved by The Rockefeller University Institutional Review Board. The subject characteristics are listed in Supplementary Table 1. DNL was measured as previously described [7].

2.3. RNA isolation and real-time PCR analysis

Total RNA from mouse livers was isolated by TRIzol extraction and purified using Zymo Research Direct-Zol™ mini columns (Zymo Research, Irvine, CA). Quantitative real-time PCR analysis was performed via standard methods using the Quantitect Reverse Transcription Kit (Qiagen, Germantown, MD) and SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA). Relative expression of mRNAs was determined after normalization to TATA box binding protein (TBP) and transformed using the equation $2^{-\Delta\Delta CT}$.

2.4. In vivo rate of hepatic lipogenesis

DNL was measured as previously described [32]. Briefly, conscious, ad libitum fed mice were injected intraperitoneally with 5 mCi of ^3H_2O and euthanized 1 h later. Once sacrificed, the liver was frozen in liquid N_2 and stored at −80 °C for processing. Lipids were extracted by the Folch method. Fatty acids were isolated by saponification and petroleum ether extraction. Incorporation of ^3H into fatty acids was measured. The rate of synthesis was calculated as a molar rate estimating 13.3 mol of H_2O per newly synthesized C16 fatty acid.

2.5. In vivo VLDL secretion

After a 4 h fast, mice were injected via the tail vain with tyloxapol (Sigma Aldrich, St. Louis, MO) at a dose of 500 mg/kg body weight. Serum was collected from tail bleeds at 0, 30, 60 and 120 min. Triglyceride levels were determined using a colormetric assay (StanBio, Boerne, TX).

2.6. Histological analysis and immunohistochemistry

A portion of the median hepatic lobe from each mouse was removed and fixed in 10% formalin at 4 °C overnight. Paraffin embedding and sectioning was performed by the Histology Core at Beth Israel Deaconess Medical Center. 5 μM sections were stained with hematoxylin and eosin or Sirius Red to visualize fibrosis.

2.7. Serum hormones and metabolites

Serum was stored at −80 °C prior to analysis. Glucose and triglycerides (StanBio, Boerne, TX) were measured using enzyme colorimetric assays. Human and mouse FGF21 were measured by ELISA (R&D Systems, Minneapolis, MN).

2.8. Hepatic triglyceride determination

Liver triglycerides were extracted using a modified Folch method. Briefly, ~100 mg of liver was homogenized in 4 ml of chloroform:methanol (2:1) and incubated overnight at room temperature. 800 μl of 0.9% saline was added. Each sample was then centrifuged at 2000 g for 10 min. The organic phase was removed and dried in a vacuum concentrator. Triglyceride content was assayed with the use of a colormetric assay (StanBio, Boerne, TX).

2.9. Statistics

All data are presented as mean ± SEM. Data sets were analyzed for statistical significance using GraphPad Prism v6.0 (GraphPad, La Jolla,

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