Brief communication

Fructose ingestion acutely stimulates circulating FGF21 levels in humans

Jody R. Dushay1,3, Elena Toschi1,3, Emilie K. Mitten1, Ffolliott M. Fisher1, Mark A. Herman1,2, Eleftheria Maratos-Flier1,2,4

ABSTRACT

Objective: Fibroblast growth factor 21 (FGF21) is a hormone with pleiotropic metabolic activities which, in rodents, is robustly regulated by fasting and ketogenic diets. In contrast, similar dietary interventions have either no or minimal effects on circulating FGF21 in humans. Moreover, no intervention or dietary challenge has been shown to acutely stimulate circulating FGF21 in either humans or animals. Recent animal data suggest that the transcription factor Carbohydrate Responsive-Element Binding Protein (ChREBP) stimulates hepatic FGF21 expression and that fructose may activate hepatic ChREBP more robustly than glucose. Here, we examined whether fructose ingestion can acutely stimulate FGF21 in humans.

Methods: We measured serum FGF21, glucose, insulin, and triglyceride levels in ten lean, healthy adults and eleven adults with the metabolic syndrome following oral ingestion of 75 g of glucose, fructose, or a combination of the two sugars.

Results: FGF21 levels rose rapidly following fructose ingestion, achieved a mean 3.4-fold increase at two hours (P < 0.01), and returned to baseline levels within five hours. In contrast, FGF21 did not increase in the first two hours following ingestion of a glucose load, although more modest increases were observed after three to four hours. Both baseline and fructose-stimulated FGF21 levels were 2–3 fold elevated in subjects with metabolic syndrome.

Conclusions: Fructose ingestion acutely and robustly increases serum FGF21 levels in humans in a pattern consistent with a hormonal response. While FGF21 appears to be critical for the adaptive response to fasting or starvation in rodents, these findings suggest that in humans, FGF21 may play an important role in fructose metabolism.

Keywords FGF21; Fructose; ChREBP; Metabolic syndrome

1. INTRODUCTION

Fibroblast growth factor 21 (FGF21) is a recently discovered hormone produced primarily by key metabolic tissues including the liver and adipose tissue [1–3] which has pleiotropic actions on glucose and lipid homeostasis [4]. Fasting or consumption of ketogenic diets markedly increases circulating FGF21 in rodent models [1,5], and FGF21 has been shown to play a key physiological role in the adaptation to starvation by enhancing hepatic fatty acid oxidation and ketogenesis [1,5,6]. Yet, fasting and ketogenic diets have little or no effect on circulating FGF21 in humans [3,7,8]. In both humans and animals, circulating levels of FGF21 are elevated in association with obesity, insulin resistance, hypertriglyceridemia, nonalcoholic fatty liver disease (NAFLD), and type 2 diabetes [3,9–12] and predict the development of the metabolic syndrome and type 2 diabetes independently of obesity [13]. FGF21 levels have also been reported to be highly elevated in humans with mitochondrial myopathies [14]. The mechanisms leading to increased FGF21 in association with cardiometabolic or mitochondrial disease are unknown. Moreover, to date no stimulus has been identified that can acutely increase serum FGF21 in either humans or animals on the time-scale of minutes to a few hours.

Recent evidence indicates that in rodents, FGF21 also responds to carbohydrate consumption and is regulated by the transcription factor Carbohydrate Responsive-Element Binding Protein (ChREBP) which is activated by products of carbohydrate metabolism [15,16]. However, acutely, an oral glucose challenge either produces no change or a decrease in circulating FGF21 [3,17]. Fructose, which is metabolized...
Brief communication

rapidly and preferentially over glucose in the liver [18], potently activates hepatic ChREBP in rodents [19]. This suggested the intriguing hypothesis that fructose ingestion might acutely stimulate production of FGF21.

We tested this hypothesis in humans by comparing the acute effects of fructose versus glucose ingestion on serum FGF21 levels. We found that fructose ingestion led to a rapid and robust increase in circulating FGF21 levels which peaked within two hours. Additionally, the FGF21 excursion following fructose was increased in subjects with metabolic syndrome. Fructose loading had no substantial effect on serum glucose or insulin levels. However, the FGF21 response to fructose correlated with indices of glucose intolerance and insulin resistance. We propose that the FGF21 response to a fructose challenge constitutes a “fructose tolerance test” and that in humans FGF21 may play an unanticipated role in fructose metabolism.

2. MATERIALS AND METHODS

2.1. Subjects

Subjects were recruited by advertisement. All study visits occurred in the Harvard Catalyst Clinical Research Center at the Beth Israel Deaconess Medical Center (BIDMC) in Boston, Massachusetts between March 2012 and September 2013. Protocols were approved by the BIDMC Institutional Review Board (ClinicalTrials.gov Identifier: NCT00968747). Screening visits included a medical history, physical examination, and baseline laboratory tests. Inclusion criteria were age 18–60 years, BMI 19–38 kg/m², no significant medical illness, and no medications except oral contraceptives. Subjects had no known history of fructose intolerance. Subjects were categorized as having the metabolic syndrome if they met criteria as defined by the National Cholesterol Education Program Adult Treatment Panel III [20].

2.2. Study protocol

After a 16 h overnight fast, subjects ingested one of the following three beverages: 75 g fructose, 75 g glucose, or a combination of 37.5 g of fructose and 37.5 g of glucose dissolved in 225 ml water. Carbohydrate beverage challenges occurred at least 2 weeks apart. Blood samples were collected every 30 min for 1 h and then every hour for 4 h after the challenge. Ten lean subjects and eleven subjects with metabolic syndrome underwent testing as described above. Four lean subjects had additional blood sampling at 90 and 150 min after fructose challenge to better define the dynamics of the FGF21 excursion following fructose ingestion.

2.3. Biochemical assays

Serum glucose, cholesterol, and triglyceride levels were analyzed in the BIDMC Clinical Laboratory according to standard procedures. Insulin samples were measured by radioimmunoassay (Millipore, Billerica, MA, USA). Serum FGF21 levels were measured using a commercially available enzyme-linked immunosorbent assay (BioVendor USA, Candler, NC) from blood collected in aprotinin-pretreated tubes. Blood samples were stored at −80 °C until use.

2.4. Calculations and statistical analyses

Data are presented as mean ± standard error. Post-ingestion hormone and metabolite values were compared to baseline values using a two-tailed paired t-test at each time point with Bonferroni correction for multiple comparisons. Comparisons of hormone and metabolite levels between lean subjects and those with metabolic syndrome were performed by two-tailed t-tests. Area under the curve (AUC) for hormones or metabolite was calculated by the trapezoidal method. Incremental area under the curve (iAUC) was calculated by subtracting the portion of AUC accounted for by the baseline metabolite or hormone level. Correlations were determined by univariate linear regression. Differences among iAUC were calculated using 2-way ANOVA and Tukey’s post-hoc test.

3. RESULTS

Baseline characteristics of the lean and metabolic syndrome study groups are summarized in Table 1. Subjects with metabolic syndrome were older and had increased BMI and waist circumference compared to lean counterparts. Metabolic syndrome subjects had lower HDL cholesterol, higher systolic blood pressure, and higher fasting and two hour glycaemia during an oral glucose tolerance test. FGF21 levels were elevated in metabolic syndrome subjects consistent with prior reports [3].

Figure 1A demonstrates the effects of a 75 g oral fructose load on serum FGF21 levels in lean, healthy subjects. FGF21 initially declined by 21% (P = 0.003) at 30 min and returned to baseline at 1 h. This was followed by a rapid, 340% increase above baseline at 2 h (P = 0.002). FGF21 levels measured in four lean subjects with more frequent sampling confirm that the FGF21 peak lies between minutes 90 and 150 (data not shown). All subjects exhibited an increase in FGF21 at 2 h of between 1.5-fold and 6.6-fold. Despite the substantial variation in the magnitude of the FGF21 response and differences in the rate of subsequent decline, serum levels returned to baseline over the subsequent 3 h in all subjects (Figure 1B).

Subjects with metabolic syndrome had higher baseline serum FGF21 levels as has been previously described [3,9,11]. Nevertheless fructose also stimulated an acute increase in serum FGF21 in these subjects. Peak FGF21 levels attained in this cohort were 2.5-fold higher (P = 0.001) than in lean subjects (Figure 2A). The FGF21 incremental area under the curve (iAUC) was 2.7-fold greater in metabolic syndrome subjects (lean: 26.2 ± 11, P = 0.002). Despite marked differences in the magnitude of the response, the kinetics were similar between lean and metabolic syndrome subjects (Figure 2A). Fructose ingestion produced a modest increase in insulin levels in both groups (Figure 2B). Fructose ingestion produced small, transient increase in glycemia (7.4 mg/dl, P < 0.05) in lean subjects at 30 min, and a slightly larger increase in glycemia in metabolic syndrome subjects (14 mg/dl at 30 min, P < 0.003; 17 mg/dl at 1 h, P < 0.005) (Figure 2C).

As fructose is lipogenic [21,22], we measured serum triglyceride levels following fructose ingestion (Figure 2D). In both lean and metabolic syndrome subjects, triglyceride levels were rapidly and preferentially increased over baseline (1.7-fold for lean and 3.2-fold for metabolic syndrome). Triglyceride levels decreased rapidly over the subsequent 2 h, with a 64% decline in lymphatic triglyceride levels in metabolic syndrome subjects compared to lean subjects (Figure 2D).
syndrome subjects, triglyceride levels tend to decline for the first 2 h and then tend to increase over the next 3 h. Triglyceride levels were higher in the metabolic syndrome subjects compared to lean subjects at 3, 4, and 5 h ($P < 0.05$).

Three lean subjects and one subject with metabolic syndrome experienced gastrointestinal symptoms (diarrhea or nausea) 30–120 min following oral fructose ingestion. The FGF21 response did not correlate with these symptoms (data not shown).

To examine the specificity of the FGF21 response, we compared the FGF21 response to fructose with the response to an equivalent amount of glucose in each subject. Previous studies had reported no increases in FGF21 levels two or three hours after glucose tolerance tests [3,17]. Consistent with this and in contrast to the acute, robust increases in FGF21 noted with fructose ingestion (Figure 2A), changes in FGF21 following glucose ingestion were more modest and delayed (Figure 2E). FGF21 levels decreased for the first hour following glucose ingestion, then rose gradually, peaked at 4 h and subsequently decreased toward baseline. Peak FGF21 levels following glucose ingestion lagged the fructose-induced peak by 2 h and were ~40% lower ($P < 0.01$) in both healthy and metabolic syndrome subjects.

FGF21 levels were higher in metabolic syndrome subjects compared to healthy controls from hours 1 through 5 following glucose ingestion (Figure 2E). Incremental AUC calculations confirm differential regulation of FGF21 by glucose versus fructose. One hundred twenty minutes after the fructose challenge, the IAUC for FGF21 was positive in both lean (7.4 ± 2 min * ng/ml) and metabolic syndrome groups (24.3 ± 3.9 min * ng/ml), and was 3-fold higher ($P = 0.002$) in metabolic syndrome subjects. In contrast, after the glucose challenge the IAUC for FGF21 was negative in both groups at 120 min (lean: $-2.5 ± 1.4$ min * ng/ml; metabolic syndrome: $-1.2 ± 2$).

As expected, glucose ingestion produced a larger increase in serum insulin and glucose levels in metabolic syndrome subjects compared to lean counterparts (Figure 2F and G). Glucose ingestion produced no significant changes in serum triglyceride levels in either healthy or metabolic syndrome subjects (Figure 2H).

The magnitude and kinetics of the serum FGF21 response following a glucose-fructose mixture are qualitatively and quantitatively similar to that seen with fructose alone (Figures 2A and 3A). Furthermore the FGF21 excursion following a fructose challenge strongly correlates with the excursion following the mixed glucose-fructose challenge (Figure 3B) ($R^2 = 0.47$, $P < 0.001$) although there is an 8 fold individual variation.

To determine whether the FGF21 response to fructose associates with impaired glucose homeostasis and insulin resistance, we examined the correlation between the FGF21 excursion following an oral fructose load with glucose and insulin excursions following a glucose load (Figure 4A and B). The FGF21 AUC following fructose ingestion correlates strongly with both the glucose ($R^2 = 0.44$, $P < 0.001$) and the insulin ($R^2 = 0.56$, $P < 0.001$) AUCs following glucose ingestion.

4. DISCUSSION AND CONCLUSIONS

This study contributes several findings relevant to the study of human metabolism. For the first time, we demonstrate that circulating FGF21 levels respond acutely to a dietary challenge — ingestion of a fructose load. Importantly, to our knowledge, this is the only known acute hormonal response to fructose ingestion. Baseline levels of FGF21 are elevated in subjects with features of metabolic disease [3,9–11,13], and we show that in this state the FGF21 response to fructose ingestion is further enhanced.

Although glucose and fructose are calorically identical they are metabolized differently [18]. Approximately 90% of an oral fructose load is extracted by the liver first pass [23]. In contrast, only a small fraction of an oral glucose load is taken up by the liver and the vast majority of glucose is disposed of in peripheral tissues [24]. These differences likely contribute to the distinct effects of glucose and fructose on hepatic lipogenesis and other metabolic processes [25]. We find that the effects of fructose and glucose on the dynamic FGF21 response are markedly different. Fructose ingestion produces a sharp, acute increase in circulating FGF21. In comparison, glucose produces a delayed and modest, but prolonged increase in circulating FGF21. These differences are likely related to the differences in hepatic fructose and glucose metabolism and support the concept that glucose and fructose ingestion may have distinct physiological consequences beyond simple caloric content [26]. Regulation of circulating FGF21 may serve as a benchmark of these differences.

The mechanisms by which fructose, and to a lesser degree glucose, increase circulating FGF21 levels remain uncertain. One molecular factor that may be involved is Carbohydrate Responsive-Element Binding Protein (ChREBP). ChREBP is master transcriptional regulator of glycolytic and lipogenic genes highly expressed in key metabolic tissues including the liver [27]. ChREBP is activated by products of carbohydrate metabolism [28], and stimulates expression of FGF21 in vitro and in animal models [15,29]. Because the liver efficiently extracts and metabolizes fructose, we hypothesized that an oral fructose challenge might acutely activate hepatic ChREBP and stimulate FGF21 production. The 60 min delay between fructose ingestion and the time when circulating FGF21 begins to rise is consistent with a
transcriptional response and the proposed mechanism. Confirming this proposed mechanism will require further investigation.

The magnitude of the FGF21 response to fructose is highly variable across subjects. Variation in the magnitude of the response may signify individual differences in fructose sensing and signaling mechanisms, including differences in rates of fructose absorption or metabolism and rates of FGF21 secretion or clearance. For example, it is known that the efficiency of intestinal fructose absorption varies widely across individuals.

Figure 2: Hormone and metabolite responses to fructose or glucose ingestion. Effects of the ingestion of 75 g of fructose on serum FGF21 (Panels A), insulin (Panels B), glucose (Panels C), and triglyceride (Panels D) in lean healthy subjects (closed circle, black line) or patients with the metabolic syndrome (open circle, gray line). Effects of the ingestion of 75 g of glucose on serum FGF21 (Panels E), insulin (Panels F), glucose (Panels G), and triglyceride (Panels H). *P < 0.05 compared to baseline within group. #P < 0.05 at given time point between groups.
individuals and approximately half the population cannot completely absorb a 25 g fructose load [30,31]. Also, the quantity of fructose in the diet can impact the ability to absorb fructose in subsequent meals [32]. We chose to administer 75 g of fructose in order to match the carbohydrate load of a standard oral glucose tolerance test although we recognize that this exceeds the amount of fructose typically ingested in a single meal. Limitations in fructose absorption can contribute to functional gastrointestinal symptoms [30,31,33]. However, few of our subjects reported symptoms and symptoms did not correlate with the FGF21 response. Although we observe large variation across individuals, the magnitude of the FGF21 response to fructose is highly correlated with the FGF21 response to a mixture of glucose and fructose within an individual. The reproducibility of this FGF21 response within subjects, but variation across subjects, is consistent with the possibility that genetics, adaptation to dietary differences, other environmental factors, or the presence of specific medical conditions such as impaired glucose metabolism might regulate the FGF21 response. Future studies are needed to further elucidate the dose-response of FGF21 to incremental oral fructose loads, to assess the impact of differences in fructose absorption on this response, and to determine whether chronic changes in fructose consumption might affect the acute response. The observation that fructose ingestion can induce an FGF21 response reminiscent of the classic response of insulin to glucose ingestion has important implications for potential physiological functions of FGF21 in humans. Based on animal studies, FGF21 was originally found to increase with fasting and regulate the starvation response [15]. However, in healthy adults, fasting does not appear to increase FGF21 in humans [3]. Several studies suggest that FGF21 may function as an endocrine signal of amino acid restriction [34–37]. In humans, 4 weeks of protein restriction increases circulating FGF21 1.7-fold [35]. These results indicate that FGF21 may play a more nuanced role in macronutrient regulation than previously suspected. Consistent with the notion that FGF21 may have unanticipated effects on macronutrient metabolism, polymorphisms in the FGF21 locus have been shown to associate with increased carbohydrate and lower fat consumption in humans [38,39]. This genetic data in conjunction with our data suggests that FGF21 might play a role in sucrose or fructose metabolism. However, a specific physiological function for FGF21 in carbohydrate metabolism remains to be identified. Both baseline and fructose-stimulated FGF21 levels are increased in subjects with metabolic syndrome. Moreover, the FGF21 response to fructose ingestion strongly correlates with the glucose and insulin excursions following glucose ingestion. This indicates that the underlying biology that determines the FGF21 response to fructose couples to the biology that defines glucose homeostasis. Expression of
Brief communication

ChREBP-β, a recently discovered potent isoform of ChREBP is elevated in the liver of people with impaired glucose metabolism [40—42]. The increased circulating FGF21 in patients with metabolic disease may therefore be due to increased hepatic ChREBP activity. In the future, it will be of interest to determine whether the FGF21 response to fructose predicts type 2 diabetes independently of more traditional risk factors and whether it might play a contributory role in the development of cardiometabolic disease.

In summary, we demonstrate that fructose ingestion acutely and robustly increases circulating FGF21 levels in humans. This response is exaggerated in subjects with metabolic syndrome and is distinct from the FGF21 response to an equivalent amount of oral glucose. The 75 g oral glucose tolerance test is the gold standard for characterizing impaired glucose homeostasis and diabetes, and the insulin response to oral glucose provides further insights into glucose homeostasis. Our findings here identify FGF21 as a measurable circulating biomarker to assess an individual’s acute hormonal response to fructose ingestion which may constitute a “fructose tolerance test.” Future studies are needed to further elucidate whether this FGF21 response might contribute to or characterize risk for the development of fructose-associated physiology or metabolic disease.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGMENTS

We thank Jeffrey Flier for insightful review of this manuscript. JRD assisted in the development of the protocol, implementation of the protocol, review of the analysis, and contributed to the manuscript writing. ET assisted in implementing the protocol, data collection, data analysis, and to the manuscript writing. EMF assisted in implementation of the protocol, data collection, and data analysis. FIM helped in implementation of the study, data collection, data analysis, and contributed to manuscript authoring. MAH and EMF supervised all aspects of the study including study design, study oversight, data analysis and manuscript authoring. This work was supported by the JPB Foundation (E.M.F.) and the Harvard Catalyst/Stanford Clinical and Translational Science Center (UL1TR000170-05 and UL1 TR001102-01). Ellison Medical Foundation is supported by R01DK028082 and MAH is supported by P30DK057521 and R01DK100425.

REFERENCES

[3] Dushay, J., Chui, P.C., Gopalakrishnan, G.S., Varela—Rey, M., Crawley, M., Fisher, F.M., et al., 2010 Aug. Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology 139(2):456—463.


