FGF21 Is a Sugar-Induced Hormone Associated with Sweet Intake and Preference in Humans

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In Brief
What is the molecular basis for a “sweet tooth”? In a combined clinical and genetic study, Søberg, Sandholt, and colleagues link the hepatokine FGF21 to increased sweet consumption in humans, potentially by acting on the central reward system.

Highlights
- Human FGF21 variants are associated with increased sweet consumption
- These variants do not correlate with obesity, T2DM, or glucose intolerance
- Circulating FGF21 is increased by sucrose consumption
- Sweet-disliking individuals have elevated FGF21 levels

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FGF21 Is a Sugar-Induced Hormone Associated with Sweet Intake and Preference in Humans

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SUMMARY

The liking and selective ingestion of palatable foods—including sweets—is biologically controlled, and dysfunction of this regulation may promote unhealthy eating, obesity, and disease. The hepatokine fibroblast growth factor 21 (FGF21) reduces sweet consumption in rodents and primates, whereas knockout of Fgf21 increases sugar consumption in mice. To investigate the relevance of these findings in humans, we genotyped variants in the FGF21 locus in participants from the Danish Inter99 cohort (n = 6,514) and examined their relationship with a detailed range of food and ingestive behaviors. This revealed statistically significant associations between FGF21 rs838133 and increased consumption of candy, as well as nominal associations with increased alcohol intake and daily smoking. Moreover, in a separate clinical study, plasma FGF21 levels increased acutely after oral sucrose ingestion and were elevated in fasted sweet-disliking individuals. These data suggest the liver may secrete hormones that influence eating behavior.

INTRODUCTION

Health is influenced by diet composition as well as total energy consumption (Mozaffarian, 2016; Solon-Biet et al., 2014). Despite this, and despite evidence in favor of independent appetites for different nutrients in model organisms, the circuits that control hunger and food-seeking in general are better understood than the interoceptive mechanisms that lead to consumption of specific nutrients, though the latter may impact both total energy intake and the health quality of food choices (Berthoud et al., 2012; de Araujo et al., 2008).

In humans, the existence of innate, nutrient-specific appetites has not been clearly established (Carreiro et al., 2016). However, food preferences are partly heritable (Pallister et al., 2015), and obesity-promoting FTO alleles have been shown to associate with elevated dietary protein intake in adults (Chu et al., 2013; Livingstone et al., 2015; Qi et al., 2014) and children (Cecil et al., 2008), raising the possibility that qualitative shifts in diet could have metabolic consequences. In addition, a fat-prefering,
sucrose-disliking phenotype was recently reported in experiments involving patients with obesity-causing mutations in the melanocortin-4 receptor, providing clinical evidence that nutrient-specific appetite is under genetic control in humans (van der Klauw et al., 2016). Finally, independent genome-wide association studies (GWASs) have correlated variants at the FGF21 locus (rs838133 and rs838145) with increased relative carbohydrate but decreased protein and fat intake (Chu et al., 2013; Tanaka et al., 2013).

An important mediator of feeding is the central reward system, which promotes adaptive actions such as consuming palatable nutrients by associating them with pleasure (de Araujo, 2011). Gastrointestinal hormones and signaling metabolites, secreted in response to nutrients in the gut, restrain further food intake in part through the reward system altering motivation to seek and consume food by influencing its reward value. Thus, efficient coupling between nutrient intake and activity of the reward system is needed to prevent overeating. For example, breakdown of this coupling due to decreased intestinal production of the fat-specific satiety factor oleoylthanolamide (OEA) contributes to the genesis of high-fat-diet-induced obesity in mice (Tellez et al., 2013). Specific negative feedback signals may also exist for sucrose and other sugars, whose consumption is promoted by both taste and reinforcing post-ingestive factors, and these signals may act directly on the reward system or indirectly by regulating production of factors like OEA that signal through afferent nerves. However, the endocrine factors that mediate sucrose satiety and reward remain unidentified (Zuker, 2015). Moreover, whether pivotal organs for sugar metabolism, such as the liver, are involved in production of these factors is unclear.

FGF21 is a liver-derived hormone that exerts a range of metabolic effects in rodents and non-human primates in both physiological and pharmacological contexts (Fisher and Maratos-Flier, 2016; Nishimura et al., 2000). Inter alia, it normalizes blood glucose in diabetic animals (Kharitonenkov et al., 2005), enhances fatty acid oxidation (Fisher et al., 2011; Potthoff et al., 2009), alleviates β cell dysfunction (Wente et al., 2006), and reduces body weight in diet-induced obese mice (Xu et al., 2009). In rodents, it also signals in the brain to regulate food intake, energy expenditure, and fertility (Laeger et al., 2014; Owen et al., 2014; Sarruf et al., 2010). However, independent clinical trials in humans (n = 38 and 50, respectively) have shown that high doses of FGF21 analogs do not lower blood glucose in obese type 2 diabetic patients, despite improving markers of insulin sensitivity, suggesting that some of its biological effects in model organisms do not extend to humans (Gaich et al., 2013; Talukdar et al., 2016b).

In rodents, plasma FGF21 increases dramatically after a 24 hr fast, ketogenic diet feeding, and dietary protein restriction (Badman et al., 2007; Inagaki et al., 2007; Laeger et al., 2014; Solon-Biet et al., 2016). In humans, however, ketogenic diet feeding and short-term fasting do not induce FGF21 (Dushay et al., 2010; Gálman et al., 2008), and 7–10 days without food are required for circulating FGF21 levels to rise (Fazeli et al., 2015; Gálman et al., 2008). In contrast, human plasma FGF21 levels are increased by several days of overfeeding irrespective of dietary protein restriction (Laeger et al., 2014; Lundsgaard et al., 2016; Maida et al., 2016), acutely by oral boluses of glucose and fructose (Dushay et al., 2014; Lin et al., 2012; Vienberg et al., 2017), and by 24 hr of hyperglycemia maintained via intravenous glucose infusion (von Holstein-Rathlou et al., 2016). Notably, the induction of FGF21 by overfeeding is associated with excess carbohydrate rather than excess fat intake (Lundsgaard et al., 2016). Because FGF21 gene variants are associated with an increased percentage of carbohydrate in the diet, together these data suggest that FGF21 could regulate nutrient-specific appetite.

Investigation of the diet composition in Fgf21 transgenic mice and cynomolgus monkeys revealed that FGF21 reduces appetite for sugars and artificial sweeteners without directly changing consumption of other nutrients or tastants, or overall calorie intake (Talukdar et al., 2016a; von Holstein-Rathlou et al., 2016). In these experiments, animals were offered a choice between a nutrient solution and water, with free access to regular chow. The ratio of nutrient to water intake between FGF21-treated and control mice was then compared to assess preference. Since FGF21 secretion by the liver is increased by simple sugars and since Fgf21 knockout mice consume more sugar than wild-type littermates, we proposed that FGF21 mediates a hormonal liver-to-brain feedback loop whereby sugar consumption negatively autoregulates sugar appetite (von Holstein-Rathlou et al., 2016). Given major species differences in FGF21 physiology, however, the significance of these findings in humans remains speculative. To address this gap in knowledge, we investigated whether FGF21 variants are associated with consumption of sucrose-rich, sweet-tasting food (broadly referred to as “sweets”) and quantitative diet composition in general in humans. In parallel, we performed a clinical study to quantify FGF21 secretion after an oral sucrose load and further tested whether the basal level or sucrose-evoked secretion of FGF21 differs between matched individuals who reported liking or disliking sweets.

RESULTS AND DISCUSSION

To investigate the relationship between FGF21 variants and human sweet appetite, we genotyped FGF21 rs838133 and rs838145 in the population-based Inter99 cohort, where detailed dietary information is available from a validated 198-item food frequency questionnaire (FFQ) (Toft et al., 2008). The two variants were in high linkage disequilibrium (r² = 0.59, D’ = 0.78) and hence produced comparable results. We analyzed the association of rs838133 with consumption of specific sweet food types. To do this, we created frequency scores summarizing weekly intake of sweet snacks between meals, grouping them into categories “candy” (sweet category, e.g., mixed candy, wine gums) and “cake” (fatty-sweet category, e.g., pastries, cake) and adding the two together to calculate the overall sweet-containing intake. The “cake” summary category we used contained all items from the original cake portion of the snack section of the validated FFQ, while the “candy” summary category also included all original items with the exception of licorice, due to its strong flavor and frequent combination with salt and ammonia in Denmark. We found that the rs838133 A-allele increased the odds ratio (OR) of being in the highest tertile of total intake of all types of sweet-tasting foods, with an OR of 1.18 per A-allele (95% CI 1.06–1.32, p = 0.003, Benjamini-Hochberg [BH] Q < 0.05)
(Table 1). When sweet intake was divided into “candy” and “cake,” we observed that individuals carrying the A-allele had higher candy intake (OR 1.19 [95% CI 1.07–1.32], p = 0.0007, BH Q < 0.05), whereas intake of cake was similar between genotype groups (p = 0.35). We also constructed a salty snack score, but found no association with rs838133 (Tables 1 and 2).

Because sweet tastant intake is regulated by the central reward system (de Araujo, 2016) and because Fgf21 transgenic mice consume less ethanol than wild-type (Talukdar et al., 2016a), a behavior that also involves the reward system, we evaluated the effect of FGF21 variants on reward-related phenotypes for which data were available from the Inter99 cohort. These analyses revealed that each rs838133 A-allele was nominally associated with higher prevalence of smoking with an OR of 1.11 for daily smoking compared to non-smoking (95% CI 1.02–1.20, p = 0.02, BH Q < 0.15), as well as increased alcohol intake (OR 1.11 [95% CI 1.02–1.22], p = 0.03, BH Q < 0.15) (Table 1). Adjustment of smoking for alcohol intake, as well as the reverse, did not change the results (Table S1). No association between FGF21 variants and coffee consumption was observed in Inter99 (p = 0.53), and the significant smoking association could not be replicated in GWAS data from the Tobacco and Genetics (TAG) Consortium (Table S2).

We then evaluated the effect of FGF21 variants on overall quantitative diet composition. Concordant with GWASs of macronutrients (Chu et al., 2013; Tanaka et al., 2013), we observed a tendency toward higher intake of carbohydrates among rs838133 A-allele carriers (p = 0.06), which was attributable to a trend to increased intake of added dietary sugars (p = 0.06), as opposed to complex (p = 0.98) or other simple carbohydrates (p = 0.13). Interestingly, there was also no difference in “fructose” intake between genotypes (Table 2). However, compared to the “added sugar” category, which is comprised primarily of sweet-tasting foods, the “fructose” category is a calculated composite that contains non-sweet items, including breads and vegetables. Intake of neither raw nor boiled vegetables varied by genotype (Table S3). We also confirmed the previously reported association with decreased protein intake among rs838133 A-carriers (p = 0.001), as well as the tendency toward decreased total fat intake (p = 0.12), mediated by decreased consumption of monounsaturated fatty acids (MUFAs) (p = 0.02), and omega-3 fatty acids (p = 0.02), and not saturated fatty acids (SFAs) (p = 0.56) (Table 2). The magnitudes of effects of these associations are consistent with published results (Chu et al., 2013; Tanaka et al., 2013). We did not find associations with intake of specific protein-containing food items (Table S3).

Because sugars are especially palatable and palatable nutrients have been implicated in the pathogenesis of obesity and metabolic disease, we also examined the association of rs838133 with anthropomorphic and metabolic variables. Surprisingly, despite the association of the rs838133 A-allele with increased intake of sweets, this allele did not correlate with increased total energy intake (Table 2) and was associated with lower BMI and waist circumference in the Inter99 cohort (Table S3), which was supported by GWAS results from the Genetic Investigation of Anthropometric Traits (GIANT) Consortium (Table S2) (Locke et al., 2015). The rs838133 A-allele carriers also tended to have better glycemic control compared with non-carriers, with lower plasma glucose or serum insulin at fasting or 2 hr after an oral glucose tolerance test (Table S3). However, the effect on glycemia was not observed in GWAS data.

Table 1. Effect of FGF21 rs838133 on Snack Intake and Reward-Related Phenotypes

<table>
<thead>
<tr>
<th>Categories, n</th>
<th>Tertile or Group</th>
<th>n</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet Snacking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total sweet snacking (3,950)</td>
<td>second</td>
<td>1,322</td>
<td>1.08</td>
<td>0.97–1.20</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>third</td>
<td>1,284</td>
<td>1.18</td>
<td>1.06–1.32</td>
<td>0.003**</td>
</tr>
<tr>
<td>Candy (4,360)</td>
<td>second</td>
<td>1,247</td>
<td>1.06</td>
<td>0.96–1.19</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>third</td>
<td>1,435</td>
<td>1.19</td>
<td>1.07–1.32</td>
<td>0.0007**</td>
</tr>
<tr>
<td>Cake (4,246)</td>
<td>second</td>
<td>1,206</td>
<td>1.00</td>
<td>0.90–1.11</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>third</td>
<td>1,373</td>
<td>1.05</td>
<td>0.95–1.16</td>
<td>0.35</td>
</tr>
<tr>
<td>Salty Snacking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total salty snacking (4,410)</td>
<td>second</td>
<td>1,425</td>
<td>0.95</td>
<td>0.86–1.05</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>third</td>
<td>1,204</td>
<td>1.05</td>
<td>0.94–1.17</td>
<td>0.41</td>
</tr>
<tr>
<td>Stimulants: Reward/Addiction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcoholic drinks (5,445)</td>
<td>second</td>
<td>2,119</td>
<td>1.08</td>
<td>0.99–1.19</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>third</td>
<td>1,910</td>
<td>1.11</td>
<td>1.01–1.22</td>
<td>0.03*</td>
</tr>
<tr>
<td>Coffee consumption (6,080)</td>
<td>second</td>
<td>2,202</td>
<td>0.98</td>
<td>0.91–1.07</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>third</td>
<td>1,490</td>
<td>1.03</td>
<td>0.94–1.13</td>
<td>0.54</td>
</tr>
<tr>
<td>Smoking (4,637)</td>
<td>occasionally</td>
<td>224</td>
<td>0.83</td>
<td>0.68–1.01</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>every day</td>
<td>2,233</td>
<td>1.11</td>
<td>1.02–1.20</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

Data represent the odds ratios (ORs) and 95% confidence interval (CI) of being in the second and third tertile of intake or consumption of the different food and reward categories. For all categories, the first tertile was set as the reference. For smoking, the OR of being occasional and every day smoker was calculated with never smoker as reference. p values (p) were calculated using multinomial logistic regression, adjusted for age and sex; asterisks denote Benjamin-Hochberg Q values; **Q < 0.05, *Q < 0.15.
was found (Table S2). The results for analysis (DIAGRAM) Consortium GWAS data (Morris et al., 2012) with type 2 diabetes in Diabetes Genetics Replication and Meta-Sortium (MAGIC) (Manning et al., 2012). Similarly, no association from Meta-analyses of Glucose and Insulin-Related Traits Consortium (MUCUFA) (% daily energy intake) 6,134 12.6 (3.4) 12.4 (3.6) 12.6 (3.7) – 0.00 (0.08) 0.98
Alcohol intake (% daily energy intake) 6,134 4.6 (5.2) 4.7 (5.4) 4.8 (5.2) – 0.10 (0.09) 0.30
Total protein (% daily energy intake) 6,134 13.9 (2.6) 13.8 (2.6) 13.6 (2.6) – 0.17 (0.05) 0.001
Total fat (% daily energy intake) 6,134 32.6 (6.9) 32.4 (7.3) 32.3 (7.4) – 0.20 (0.13) 0.12
MUFA (% daily energy intake) 6,134 10.8 (2.7) 10.7 (2.9) 10.6 (2.8) – 0.09 (0.05) 0.08
PUFA (% daily energy intake) 6,134 5.0 (1.5) 5.0 (1.5) 4.8 (1.5) – 0.06 (0.03) 0.02
SFA (% daily energy intake) 6,134 12.6 (3.4) 12.4 (3.6) 12.6 (3.7) – 0.04 (0.06) 0.56
Omega-3 fatty acids (% daily energy intake) 6,134 0.9 (0.3) 0.9 (0.3) 0.9 (0.3) – 0.009 (0.006) 0.08
Food Item Frequencies

<table>
<thead>
<tr>
<th>Food Item</th>
<th>n</th>
<th>WT (GG)</th>
<th>HE (GA)</th>
<th>HO (AA)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sweet snacking (servings/week)</td>
<td>3,950</td>
<td>4.9 (2.6;8.1)</td>
<td>5.0 (2.8;8.5)</td>
<td>5.8 (3.1;9.1)</td>
<td>– 0.02</td>
</tr>
<tr>
<td>Candy intake (servings/week)</td>
<td>4,360</td>
<td>2.5 (1.3;4.5)</td>
<td>2.6 (1.3;4.8)</td>
<td>3.0 (1.5;5.2)</td>
<td>– 0.04</td>
</tr>
<tr>
<td>Cake intake (servings/week)</td>
<td>4,246</td>
<td>1.8 (0.9;3.6)</td>
<td>1.8 (0.9;3.6)</td>
<td>1.9 (0.9;3.9)</td>
<td>– 0.07</td>
</tr>
<tr>
<td>Salty-snacking (servings/week)</td>
<td>4,410</td>
<td>0.9 (0.5;1.8)</td>
<td>0.9 (0.5;1.8)</td>
<td>1.1 (0.3;1.9)</td>
<td>– 0.39</td>
</tr>
<tr>
<td>Fructose intake (servings/week)</td>
<td>6,134</td>
<td>8.0 (4.5;16.3)</td>
<td>8.3 (4.3;17.0)</td>
<td>8.3 (4.4;16.1)</td>
<td>– 0.44</td>
</tr>
<tr>
<td>Complex carbohydrates (servings/week)</td>
<td>3,014</td>
<td>39.7 (30.2;49.8)</td>
<td>38.7 (28.8;48.8)</td>
<td>40.4 (30.2;50.5)</td>
<td>– 0.94</td>
</tr>
</tbody>
</table>

Quantitative diet outcomes are given as mean ± SD, except for added sugar and food item frequencies, which are given as median ± interquartile range. p values and per allele effect sizes (β) were calculated using general linear models assuming an additive genetic model (p trend), adjusted for age and gender. MUFA, mono-unsaturated fatty acid; PUFA, poly-unsaturated fatty acid; SFA, saturated fatty acid.

Table 2. Association between FGF21 rs838133 and Quantitative Dietary Outcomes

from Meta-analyses of Glucose and Insulin-Related Traits Consortium (MAGIC) (Manning et al., 2012). Similarly, no association with type 2 diabetes in Diabetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium GWAS data (Morriss et al., 2012) was found (Table S2). The results for FGF21 rs838145 were highly consistent with those for rs838133 (Table S4).

In a separate clinical study, we investigated the association between fasting plasma FGF21 levels and self-reported sweet liking in young, healthy, and lean subjects. A total of 86 subjects (23 men and 63 women) completed a questionnaire to determine sweet, fatty-sweet, and salt preferences (Deglaire et al., 2012). Based on sweet-liking scores, we selected the 51 subjects from the highest (n = 25, 19 women and 6 men) and lowest (n = 26, 19 women and 7 men) tertiles of sweet preference and classified them as “sweet-likers” and “sweet-dislikers,” respectively. Although more women than men participated in this study overall, there was no difference in the sex distribution between the two groups. Prior to blood sampling for FGF21 analysis, we also asked each subject to select images of liked and disliked snacks. We measured FGF21 levels after a 12 hr fast and found that concentrations were 51% higher in sweet-dislikers compared to sweet-likers (median 95.7 pg/mL [interquartile range, 65.5–422.2] versus median 63.2 pg/mL [interquartile range, 51.5–108.8], p = 0.04) (Table 3).

We measured FGF21 levels after a 12 hr fast and found that concentrations were 51% higher in sweet-dislikers compared to sweet-likers (median 95.7 pg/mL [interquartile range, 65.5–422.2] versus median 63.2 pg/mL [interquartile range, 51.5–108.8], p = 0.04) (Table 3).

On a subsequent clinical visit in 41 of the 51 subjects (20 sweet-likers and 21 sweet-dislikers recalled randomly from each group on the basis of a power calculation), we performed a 5 hr, 75 g oral sucrose challenge following a 12 hr fast to assess the dynamic FGF21 response to sucrose consumption and to investigate potential differences in FGF21 secretion between sweet-likers and sweet-dislikers. In the group that completed the challenge study, there was no difference in absolute plasma FGF21 levels between sweet-likers and sweet-dislikers at any time point, and incremental areas under the curve were indistinguishable between groups (p = 0.26). Most importantly, however, plasma FGF21 was markedly increased by sucrose (mean 193% above baseline, p < 0.0001) in both groups, reaching maximum levels after 120 min (Figures 1A and 1B, respectively). Interestingly, this

...
increase was delayed relative to the increase in plasma insulin, plasma C-peptide, and plasma glucose, which peaked 30–60 min after the oral sucrose challenge (Figures S2A–S2F; Table S6). The large variation in inter-subject FGF21 levels is also notable and consistent with prior reports (Gálman et al., 2008). 

The mechanisms that influence what foods humans want to consume may contribute to the global burden of morbidity and premature death because suboptimal diet is a primary risk factor for many common diseases (Mozaffarian, 2016). The ubiquity of palatable nutrients, especially sugars and fat, contributes to poor dietary choices by exploiting the tendency, which likely evolved in response to frequent periods of scarcity, to voraciously consume such energy-dense foods when available (Yarmolinsky et al., 2009). Thus, a better understanding of the biological basis of palatable nutrient appetite is needed to develop strategies to improve diet quality and human health in modern food environments (Zuker, 2015). 

Our data suggest that the liver hormone FGF21 may regulate sweet consumption in humans, offering insight into the fundamental biology of nutrient appetite as well as a potential avenue for developing therapeutics to decrease intake. Consistent with this hypothesis, two GWASs have associated SNPs in FGF21 with relatively increased intake of carbohydrate but decreased intake of protein and fat in humans (Chu et al., 2013; Tanaka et al., 2013), and a recent paper reported that FGF21 variants interact with diet to modify changes in fat mass and waist circumference evoked by weight loss diets containing either high or low amounts of carbohydrate (Heianza et al., 2016). However, the former reports do not address how FGF21 variation modifies diet pattern beyond basic relative macronutrient consumption. This study, by contrast, explores how these FGF21 variants correlate with intake of many food items from diverse categories, to produce a detailed portrait of how FGF21 variation affects human ingestive behavior. We show that the rs838133 A-allele and highly correlated rs838145 G-allele associate specifically with increased total intake of sugars rather than complex carbohydrates, as well as the propensity to consume sweet snacks rather than fatty-sweet or salty snacks. Importantly, this change in diet structure does not affect total energy intake, as both protein and fat intake decrease. This is consistent with previous observations in rodents of a specific effect of FGF21 on sweet consumption, which leads to compensatory changes in protein and fat intake to maintain stable energy intake (von Holstein-Rathlou et al., 2016). 

It is notable that the strongest association observed in our study was with sweet snacking, candy in particular. Snacking...

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**Table 3. Clinical Measures among Sweet-Likers and Sweet-Dislikers**

<table>
<thead>
<tr>
<th></th>
<th>Sweet-Likers</th>
<th>Sweet-Dislikers</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (women/men)</td>
<td>25 (19/6)</td>
<td>26 (19/7)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>23.7 (2.6)</td>
<td>24.4 (3.5)</td>
<td>0.39</td>
</tr>
<tr>
<td>Body mass index (BMI) (kg/m²)</td>
<td>21.9 (1.7)</td>
<td>21.8 (1.6)</td>
<td>0.70</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>76.3 (6.6)</td>
<td>75.6 (3.9)</td>
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<td>Fasting plasma glucose (mmol/L)</td>
<td>4.7 (0.4)</td>
<td>4.6 (0.4)</td>
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<td>5.1 (0.7)</td>
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<td>Fasting plasma FGF21 (pg/ml)</td>
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<td>64.4 (51.5;286.5)</td>
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Data are given as median ± interquartile range, with differences between groups assessed using a Wilcoxon rank test (p). 

A Data are given as mean ± SD

B Differences between groups are evaluated using a Student’s t test

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**Figure 1. Changes and Concentrations of Plasma FGF21 during an Oral Sucrose Challenge Test**

(A) The percent changes in plasma FGF21 levels in each subject from baseline at different time points during an oral sucrose challenge. Data presented as mean ± SEM.

(B) The absolute concentrations of plasma FGF21 at different time points during an oral sucrose challenge. Data presented as median ± interquartile range. The circles and blue line represent sweet-likers (n = 20), and the squares and green line represent sweet-dislikers (n = 21).
often occurs in the absence of energy deficit, motivated by a desire for pleasure. Thus, FGF21 may regulate hedonic sugar craving, as opposed to homeostatic sugar appetite. This possibility is congruent with the potential association between the rs838133 A-allele and other forms of reward-seeking behavior. The connection to alcohol is of particular interest because Fgf21 transgenic mice are alcohol averse (Talukdar et al., 2016a), and a recent GWAS involving more than 105,000 individuals identified variation in the obligate FGF21 co-receptor, β-klotho, as associated with alcohol drinking in humans (Schumann et al., 2016). Thus, alcohol, like glucose and fructose, may increase FGF21 production to limit further alcohol intake, although more studies are needed to verify this hypothesis.

Our finding that the rs838133 A-allele, which correlates with increased intake of sweets, is associated with decreased BMI and waist circumference in the Inter99 cohort and GIANT consortium, suggests that small increases in sugar consumption do not necessarily promote adiposity or glucose intolerance in humans. Instead, if a shift in diet composition underlies these effects, it suggests that reducing intake of protein or certain fats may have beneficial effects on whole-body energy balance. Consistent with this, the FTO rs1421085 C-allele is associated both with increased BMI and increased protein intake (Tanaka et al., 2013). Interestingly, the FGF21 rs838133 A-allele is also associated with increased plasma homocysteine levels, but not coronary artery disease, in a sample of 31,400 cases and 92,927 controls (van Meurs et al., 2013). However, the rs838133 A-allele is nominally associated with an increased risk of ischemic stroke (OR = 1.04), small vessel disease (OR = 1.08), and large vessel disease (OR = 1.09), but not cardioembolic stroke, in 12,389 cases and 62,004 controls (Gotliciuc et al., 2014). Thus, our data suggest that while increased sugar consumption is not associated with obesity, it may correlate with adverse vascular outcomes. Based on these results, we are hopeful that improved methods of capturing human dietary behavior can be combined with GWASs to infer how long-term eating patterns influence body weight and other cardiovascular outcomes, a task that has been difficult and controversial to date.

Our clinical trial also suggests that human FGF21 may be a negative regulator of sweet consumption because it increased markedly after an oral sucrose load and because sweet-disliking individuals have elevated fasting FGF21 levels. Interestingly, taste ratings of sucrose intensity and pleasantness did not differ between groups. This observation is in agreement with a previous report demonstrating that FGF21 increases with a hormone-like profile in humans after oral fructose and glucose ingestion, as well as data from rodents and non-human primates showing that FGF21 treatment reduces sweet consumption independent of taste (Dushay et al., 2014). However, the rs838133 A-allele, which is associated with increased consumption of sweets, is highly correlated with the rs838145 G-allele, which was associated with higher fasting plasma levels of FGF21 in 377 samples from the Baltimore Longitudinal Study of Aging (Tanaka et al., 2013). The explanation for this is likely that the kit used by Tanaka et al. (R&D Systems) and the kit used in our clinical study to measure total FGF21 (Biovendor) exhibit poor intra-subject correlations in the fasted state for reasons that are unknown, but may involve use of a monoclonal capture antibody in the R&D Systems kit that cannot detect cleaved forms of FGF21, or some interference in the Biovendor kit that leads some subjects to exhibit extremely high FGF21 levels (see STAR Methods for technical discussion of this issue). However, both kits give similar conclusions about the magnitude of FGF21 increase after sucrose ingestion, which are also congruent with results from ELISA reagents that only measure full-length FGF21, suggesting both detect relative changes in FGF21 similarly (data not shown). Studies that are more detailed will be required to understand this measurement discrepancy in fasting samples.

The major strength of this study is that it combines human genetics and clinical investigation to test GWAS-inspired results from rodents and primates again in humans. This species back and forth allowed us to evaluate a narrow hypothesis of high prior probability about a potential function of FGF21 in humans and conclude that a clinical trial examining whether FGF21 influences sweet intake, alcohol drinking, and smoking is warranted. The major limitations of this study are its reliance on self-reporting and that it does not experimentally demonstrate that FGF21 regulates sweet appetite and reward seeking, an outcome that awaits future interventional trials.

**Conclusion**

Our results indicate that circulating FGF21 may regulate sweet intake in adult humans, consistent with its effects in mice and non-human primates. Conceptually, our findings suggest that the human liver might produce hormones that regulate food intake by acting on the reward system, opening a new front in the search for therapeutics to reduce appetite and modify specific food intake.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and six tables and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2017.04.009.

AUTHOR CONTRIBUTIONS

S.S. and C.H.S. designed and performed experiments, interpreted data, and wrote the first draft of the paper. U.T., S.v.H.-R., and T.J.G. designed and performed experiments and interpreted data. N.Z.J., K.B.C., W.L.P.B., T.P.J.S., M.J.P., A.L.M., A.L., T.J., O.P., T.H., and C.S. designed experiments, oversaw data collection, interpreted data, and contributed essential reagents or expertise. N.G. and M.P.G. conceived of the project, designed experiments, oversaw data collection, interpreted data, wrote the paper, and are responsible for the integrity of its content. All authors contributed to, edited, and approved the manuscript prior to submission.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING CITATIONS

The following references appear in the Supplemental Information: Shungin et al. (2015); Tobacco and Genetics Consortium (2010).

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Dushay, J., Chui, P.C., Gopalakrishnan, G.S., Varela-Rey, M., Crawley, M., Fisher, F.M., Badman, M.K., Martinez-Chantar, M.L., and Maratos-Flier, E. (2010). Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology 139, 456–463.


STAR METHODS

KEY RESOURCES TABLE

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CONTACT FOR REAGENT AND RESOURCE SHARING

Requests for further information or data should be directed to and will be fulfilled by the Lead Contact, Matthew Gillum (gillum@sund.ku.dk).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The Inter99 study

Genetic association studies were performed in the Danish population-based Inter99 study (ClinicalTrials.gov ID-no: NCT00289237), which is a non-pharmacological intervention study for ischemic heart disease performed at the Research Centre for Prevention and Health, Glostrup, Denmark (Jørgensen et al., 2003). A random sample of 13,016 individuals living in Copenhagen County from seven different age groups (30-60 years, grouped with five year intervals) was drawn from the Civil Registration System and 6,784 of these attended the health examination (Jørgensen et al., 2003). All participants were Danes by self-report, and 6,514 individuals were eligible for genetic analyses. At the health examination, anthropometric data were collected and biochemical analyses were performed. The latter included measurements of lipid profile (serum total cholesterol, triglyceride, high density lipoprotein (HDL)-cholesterol) and glucose homeostasis (plasma glucose and serum insulin levels, after fasting and during an oral glucose tolerance test (OGTT), with measurements taken at 30 and 120 min).

Recruitment of participants for clinical study

Men and women between 18-39 years of age, with a BMI between 19-25 kg/m\(^2\) and without any medical conditions, were recruited for participation by advertisement at the University of Copenhagen and a webpage for volunteers for clinical studies (www.forsøgsperson.dk). Individuals with chronic diseases, history of alcohol abuse, and smoking were excluded. Additionally, individuals dieting, taking dietary supplements, with recent weight loss (> 3 kg within 3 months), a history of eating disorders, lactation within 12 months prior the study or use of daily medications (except users of anti-contraceptive pills or seasonal use of antihistamines) were excluded for participation. The study was approved by the Scientific Ethics Committee of the Capital Region of Denmark (H-1-5004-650). All individuals provided written informed consent prior to participation in the study, which was performed in accordance with the principles embodied in the Declaration of Helsinki II.

METHOD DETAILS

Genotyping

The two FGF21 SNPs (rs838133 and rs838145) were genotyped by LGC Genomics, UK, using the KASP genotyping technique. The success rates were > 98.8% and the error rate < 0.38% (based on > 538 duplicates), and Hardy-Weinberg equilibrium were obeyed for both SNPs (p > 0.025).

Inter99 study methods

Detailed information on lifestyle was collected using questionnaires, including a detailed, validated semiquantitative 198-item FFQ (Toft et al., 2008). Specific dietary measures, such as a profile of macronutrients (total carbohydrates, simple carbohydrates, complex carbohydrates, fats, etc.), were obtained using questionnaires. The FFQ contained questions about the frequency and portion sizes of various food items consumed over the past year. The data were analyzed using statistical software, and the results were reported in the form of averages and standard deviations.
carbohydrates, protein, total fat, saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), and omega-3 fatty acids) comprising the daily energy intake (in percentages) were retrieved from the FFQ using the software program FoodCalc version 1.3 linked with the Danish Food Composition Databank version 6. The number of servings per week of selected food items was also recorded, including: candy, cake, total sweets (candy plus cake), salty snacks, and foods with a high content of complex carbohydrates. A health and lifestyle questionnaire additionally provided information regarding alcohol intake (units per week), coffee consumption (cups per day), and smoking habits. The latter divided the individuals into four categories i) never smoked, ii) former smoker, iii) occasional smoker, and iv) daily smoker. Alcohol intake was analyzed as a summary score (normal beer, strong beer, glass of red wine, glass of white wine, spirits).

Dietary and reward/addiction outcomes

The food frequency questionnaire (FFQ) used in the Inter99 study was based on diet recall within the last month. It contained questions regarding different food items at all meals during the day, and answers stated the intake frequencies (per month if appropriate, per week if appropriate, or per day if appropriate). It was based on validated FFQs modified to improve the intake estimates of specific fatty acids such as saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), omega-3 fatty acids, as well as cholesterol and complex carbohydrates. This resulted in a 198-item FFQ, which was validated using diet-history records and biomarker analysis (Toft et al., 2008). The FFQ was used to estimate daily energy intake (kJ), macronutrient intake including carbohydrates, protein, and fat (as percentages of daily energy intake), as well as micronutrient intake such as specific vitamins and minerals (Toft et al., 2008). Calculations were done using the software program FoodCalc version 1.3 (Lauritsen, 1998) and information from the Danish Food Composition Databank version 6 (http://www.foodcomp.dk/v6/fvdb_default.asp).

In the present study we constructed specific scores of sweet and salty-snacking as well as food items with high content of fructose and complex carbohydrates (servings/week). Sweet-snacking scores were constructed summarizing servings of i) candy (ice cream, chocolate, wine gum, mixed candy, other types of candy) and ii) cake (cake, Danish pastry, cream cakes, cookies and crackers, other types of cake), and the total intake of sweet snacks was estimated adding the two together. The score comprising food items with high content of fructose summarized servings of fresh fruit, dried fruit, preserved fruit, and fruit spreads (for bread, however, not jam). The scores comprising food items with high content of complex carbohydrates summarized servings of rye bread, white bread, whole meal bread, Italian bread, rolls, buns, crispbread, other types of bread, boiled potatoes, baked potatoes, mashed potatoes, pasta, and rice.

To elaborate on the association between rs838133 and protein intake we addressed whether the variant associates with specific food preferences such as different meat types or the intake of vegetables. To do this we calculated scores summarizing the intake of; i) cold meat on the sandwiches, often rather processed meat (liver pate, salami, pork sausage, rolled seasoned meat, ham, roast beef, smoked/salted pork filet, chicken or turkey breast, roast pork), ii) minced meat dishes (meat balls, minced beef steak, veal/pork patty, sausages, minced meat stews, meat loaf, lasagne) iii) poultry (chicken, turkey, ostrich, duck, goose, pheasant, stews with poultry). Moreover, we summarized the intake of different cuts or stews of various meat types including: i) beef and veal ii) pork and iii) lamb. The intake of raw and boiled vegetables was assessed in the FFQ as overall servings/week, hence intake of specific types of vegetables could not be addressed.

Due to accumulation of individuals in the low end of the food item scores, they were divided into tertiles before statistical analyses. The 1st tertile was used as a reference to test effect of the FGF21 SNPs on the odds of being in the 2nd and 3rd tertile. The reward or addiction phenotypes of alcohol intake, coffee consumption, and smoking were retrieved from the health and lifestyle questionnaire also filled out by all Inter99 participants. Alcohol intake was analyzed as a summary score (normal beer, strong beer, glass of red wine, glass of white wine, spirits). Coffee consumption was stated as cups of coffee per day. The question regarding smoking habits divided the individuals into four categories i) never smoked, ii) former smoker, iii) occasional smoker, and iv) daily smoker. In the present study never smoked was used as a reference to test the effect of the FGF21 single nucleotide polymorphisms (SNPs) on the odds of being occasional and daily smoker. Alcohol intake and coffee consumption was divided into tertiles, and the 1st tertile was used as a reference to test effect of the FGF21 SNPs on the odds of being in the 2nd and 3rd tertile.

Biochemical measurements for the Inter99 study

Blood samples were drawn after a 12 hr overnight fast. Plasma glucose was analyzed by glucose oxidase method (Granustest; Merck, Darmstadt, Germany) and serum insulin (excluding des(31,32) and intact proinsulin) was measured using the AutoDELFIA insulin kit (Perkin-Elmer, Wallac, Turku, Finland). Serum triglyceride, total cholesterol, and HDL-cholesterol were determined using enzymatic calorimetric methods (GPO-PAP and CHOD-PAP; Roche Molecular Biochemicals, Mannheim, Germany).

Clinical study methods

In total, 86 subjects (23 men and 63 women) filled out a translation of the validated French PrefQuest questionnaire. The PrefQuest differentiates between the taste sensations; sweet, fatty-sweet, fatty-salt, and salt, and consists of 143 items divided into four sections about food preferences, including the preferred levels of seasoning, preferred dishes on a menu, and dietary behavior (example in Figure S1) (Deglaire et al., 2012). To minimize differences from the original questionnaire, the same layout, colors and pictures were used; hence, visually only the language was different from the original. However, 12 items were replaced to match Danish eating habits. Additional items were only added to the questionnaire for validation of the grouping. However, these were not used in calculating the sweet-liking composite score and were analyzed independently. Among these responders, 5 were excluded due to dieting, occasionally smoking or extreme answers on more than 25% of items regarding the same taste sensation, as was previously
done (Deglaire et al., 2012). The sweet-liking score retrieved from this questionnaire was used to select the participants for the study (n = 51). Those in the highest (4.1–5.7) and lowest (2.5–3.5) quartiles by score were defined as sweet-likers (n = 25) and sweet-dislikers (n = 26), respectively. More women than men answered the questionnaire; however the two groups were matched ensuring equal sex distribution.

The subjects selected for the study underwent pre-examination that included baseline laboratory tests (after a 12 hr overnight fast), medical history and physical examination. Female subjects were pregnancy tested using plasma HCG measurements, to be excluded if positive. A lower self-rating of “sweet tooth” was observed among sweet-dislikers compared to sweet-likers (p = 0.005) (Figure S1C).

A 5 hr sucrose challenge test was performed within 2 days to 3 weeks after the pre-examination. Subjects were instructed to refrain from alcohol intake 48 hr and fast for 12 hr (overnight) prior to participation. Blood samples were collected in the fasting state, as well as 30, 60, 120, 180, 240, and 300 min after ingestion of a solution containing 75 g sucrose dissolved in 225 mL tap water. Based on a power calculation, 41 randomly selected subjects of the original 51 subjects participated in the sucrose challenge test.

Sweet preference questionnaire details

The questionnaire used for selection of study participants is a French validated questionnaire specifically developed to determine food/taste preferences (Deglaire et al., 2012), translated into Danish to be used in a Danish population. Overall, the questionnaire contained four sections to get a detailed determination of food/taste preferences; i) Liking of foods, where participants were asked to rate their liking for a given food on a nine-point scale. If the participant had never tasted the food in question, he/she could choose “I have never tasted” as an answer. ii) Preferred level of seasoning, where participants were asked to give their preferred level of seasoning for a given food on a 6-point scale. The scale points 0, 2, and 4 were illustrated by a picture (Figure S1) of the same food with grading levels of salt for the salt scale (nine items); mayonnaise, butter, cream or grated cheese for the fat-and-salt scale (13 items); sugar or jam for the sweet scale (nine items), and whipped cream, chocolate spread (Nutella), or butter for the fat-and-sweet scale (10 items). If the respondents did not like the food in question, he/she could choose “I do not like [this food]” as an answer.

Eight distracting items related to other seasonings, e.g., lemon, ketchup, and pepper were randomly included and were not included in the composite scores. The effect of the illustrative pictures were assessed by including 12 duplicate questions (3 in each scale) asking about the preferred level of seasoning but with no picture, with a 5-point scale labeled at each anchor, and with the additional “I do not like [this food]”. iii) Preferred dishes in a menu, where participants were told that a new restaurant was about to open in their neighborhood and that, beforehand, a survey was organized about the food preferences of the future clientele. The participants had to choose the dishes they found the most tempting within each list, with a maximum number of three out of an eight-dish list or two out of a six-dish list. They could answer that none of the dishes attracted them. For each type of dish usually proposed in a restaurant, a list of six or eight dishes or drinks was established that were more or less salty (e.g., cocktail snacks and meat), fatty and salty (e.g., meat, side dishes and Italian food), sweet (appetizer drinks, desserts and soft drinks), and fatty and sweet (desserts, snack desserts, and hot drinks). iv) Sweet, salt, or fat-related dietary behavior questions, where a five-point frequency scale or a nine-point scale was used, depending on the item. If applicable, an additional point saying “I have never tasted” or “I do not like [this food]” was included. There were 6 items for salt-scale, 3 for the fat-and-salt scale, 4 for the sweet scale, and 4 for the fat-and-sweet scale.

The French and Danish cuisines can be very similar, however, a few items in the French version was replaced to match the Danish eating habits e.g., turkey was replaced with chicken. In total 12 items were replaced, and seven fatty and five fatty-sweet items were removed. Only one of the changed items involved the sweet scale in section III, however, the changes in the questionnaire were in general relatively few. All in all our translated Danish version contained 12 items for salt, 37 items for fatty-salt, 39 items for sweet, 39 items for fatty-sweet, 10 distracting items, and three behavioral items. In total 140 items translated from the French questionnaire.

Calculation of food preference scores

Data was transformed for analysis, as previously done in the original French questionnaire (Deglaire et al., 2012), meaning that data for section I, II, and IV were linearly transformed into values ranging from 0 to 10. For each scale, answers to the additional point labeled “I have never tasted” or “I do not like [this food]” were estimated by adding the mean of all the ratings of a given individual to the mean of the ratings of a given item, and then subtracting the mean of the entire sensation. Participants who answered “I have never tasted” or “I do not like [this food]” to more than 25% of items within a sensation were not considered for further analysis. For questions in section III, each dish list was calculated as a rating by dividing the number of dishes considered as salty, fatty-salty, sweet, or fatty-sweet, by the total number of dishes selected in the list. If a participant answered that none of the dishes attracted them, a rating of 0 was attributed. In total, 86 individuals (23 men and 63 women) filled out the questionnaires, and participants were selected by their composite sweet liking scores for the experiments. The lowest tertile of sweet liking scores corresponded to the group of sweet-dislikers (n = 26) and the highest tertile of sweet liking scores as the group of sweet-likers (n = 25).

Added items to the questionnaire

Besides a translated version of the French questionnaire, additional items were added. In previous studies, it has been shown that people are able to rank their own degree of “sweet tooth.” Adding an extra self-rated “sweet tooth item” with an additional “soda
consumption item” provided a psychological dimension in the degree of self-awareness of the body and its signals. In the “sweet tooth” rating, participants could answer: i) a lot, ii) moderate, iii) a little, or iv) none. In the soda consumption item, responders were asked to rate soda consumption during the last couple of months rating from none to three or more times a day. Finally, a dichotomous answer was added asking “What do you prefer: Salty or Sweet”? Additionally, questions regarding the basic characteristics such as age, occupation, smoking status and amount of physical activity were added at the end of the questionnaire.

The three additional food item questions were not included in the sweet composite score for sweet liking and were therefore analyzed independently. However, a lower self-rating of “sweet tooth” was in fact observed among sweet-dislikers compared to sweet-likers (p = 0.005) (Figure S1).

The added question regarding soda consumption showed that a total 45% of participants drink soda less than once a week, 12% never drink soda, 25% less than once a month, 12% once a week, 4% every other day and only 2% once a day. No participants claimed to drink soda twice or more a day. Consumption of soda was similar between sweet-dislikers and sweet-likers (Mean: 1.65 (±1.23) versus 1.88 (±0.88) respectively, p = 0.45).

In the rating of liking for sweet or salt, 43% of sweet-dislikers and 44% of sweet-likers preferred sweet over salt. 57% versus 56%, respectively, answered to prefer salt over sweet (p = 0.97 between groups).

Snack-preference test
As the online questionnaire was filled out prior to the actual pre-examination (one to three weeks before), we moreover, performed a snack-preference test to verify that sweet-dislikers still preferred less sweet snacks compared to sweet-likers. Sixteen neutral pictures of non-branded snack food categories were presented on a table for each participant. The pictures represented the five taste senses; sweet (six pictures), sour (two pictures), bitter (three pictures), salt (three pictures), and umami (two pictures). Each picture showed one item on a neutral background. Participants were asked to select three pictures that represented the snack-categories they liked the best and three pictures representing the least liked/disliked snacks. All participants had the same instruction before addressing the test. The snack-preference test revealed that sweet-dislikers disliked more sweet snacks than sweet-likers (defined as the choosing of more pictures of sweet snacks that they disliked) (p = 0.02) and the other way around that sweet-likers preferred more sweet snacks compared to sweet-dislikers (defined as the choosing of more pictures with sweet snacks they liked) (p = 0.04) (Table S5).

Sweet taste perception test
In order to determine the perception of sweet taste in the two pre-defined groups of sweet preference in the clinical study a direct measure of sweet taste was performed. Five different sucrose concentrations were used as done previous in similar experiments (Laeng et al., 1993; Sartor et al., 2011). Each solution contained a constant amount of a commercial soft-drink concentration (Rynkeby). For a ‘base’ we use 180 mL soft drink concentration per liter tap water. The base was used to make five different concentrations by adding varying amounts of sucrose. All five solutions contained added sugar. To make the first solution we added 30 g of sugar to the base to make the solutions as equal in taste as possible by having added sucrose to every solution. The next concentration contained the double amount of sugar.

The five concentrations were presented in random order and labeled with undetectable codenames, as previously done (Laeng et al., 1993; Sartor et al., 2011) (random three digit numbers as codes; omitting codes with particular meaning, e.g., 007). Subjects were not allowed to see the bottles containing the solutions and drinks were presented in opaque plastic cups in order to reduce visual cues about the viscosity differences. The solutions were made and used at the same day and contained at 4°C in a refrigerator until needed. Sweetness intensity and liking of sweet taste was assessed by a sip-and-spit procedure (Laeng et al., 1993; Sartor et al., 2011). Subjects spit out “the sip” to avoid the satiety of the sugar and thereby avoid biased ratings in the next solution. Each drink contained 10 ml. To avoid effects of adaptation and thus response bias before and between tasting each concentration, the subjects actively rinsed their mouths with 40-50 mL of tap water and waited for 2 min between each tasting trial. Before the test all subjects were orally given the same instructions about the test and the ratings. When tasting the solutions, the subjects were instructed to use a sufficient amount of the solution given to guarantee exposing all the taste buds in the oral cavity for at least 10 s before they were allowed to spit out the solution. The solutions were tested in order of increasing concentration and the subjects were asked to identify the intensity level and second they were asked to identify the liking of the solution using a Visual Analog Scale from 1 = low intense to 9 = high intense and then the liking of the solutions from 1 = Extremely dis-like to 9 = extremely like it, with 5 value as indifferent. The scales were presented visually on paper and subjects were asked to mark the selected value with a pen after each solution. The taste-test was repeated three times with 5 min break in between to avoid the satiety of the previously round.

Sucrose challenge test
Two days to three weeks after the pre-examination a 5 hr sucrose challenge test was performed. Participants were told not to drink alcohol 48 hr prior to participation and arrived at the examination day after a 12 hr overnight fast. Blood samples were collected in the fasting state, as well as 30, 60, 120, 180, 240, and 300 min. after ingestion of a solution containing 75 g sucrose dissolved in 225 mL tap water.

Based on a power calculation, of the 51 individuals selected based on the sweet liking score, 41 participants completed the sucrose challenge test (n = 21 sweet-dislikers and n = 20 sweet-likers).
**Biochemical measures for clinical study**

Blood samples used for FGF21 measurements were immediately put on ice and separated by centrifugation for 15 min at 3000 x g. Plasma samples were subsequently stored in aliquots at −20 to −80°C until further analysis. Plasma FGF21 levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (BioVendor, Brno, Czech Republic). The standard curve range for the assay was 7–1900 pg/ml. FGF21 was assayed in duplicate or triplicate and calculated using the mean replicate value.

**General hormone analyses and assay validation studies**

A pre-examination with routine blood-samples was performed on all 51 subjects to test for diseases before they were included in the experiments. The circulating levels of plasma insulin, plasma C-peptide, plasma glucose, plasma cholesterol, plasma triglycerides, plasma alanine transaminase (ALAT), plasma aspartate transaminase (ASAT), plasma thyroid hormones, hemoglobin in blood, glycosylated hemoglobin (HbA1c), plasma thyrotropin, plasma alkaline phosphatase, plasma carbamide, and plasma urea in blood were measured using standard techniques at the Department of Clinical Biochemistry, Rigshospitalet University Hospital, Copenhagen, Denmark.

Blood samples for insulin, C-peptide and FGF21 were immediately spun at 4°C at 3000 g for 15 min and the plasma fractions for FGF21 were stored at −80°C until analysis. On each experimental day, C-peptide, insulin, and glucose samples were stored at 2-4°C until analyzed later the same day. Plasma insulin was analyzed by electrochemiluminescent immunoassay (Cobas; Roche) and C-peptide by Sandwich electrochemiluminescence-immunoassay (ECLIA). Two commercial kits are commonly used to measure total FGF21 in human plasma, one from R&D Systems (Vendor A) and the other from Biovendor (Vendor B) (Bobbert et al., 2013; Hanssen et al., 2015; Mraz et al., 2009; Zhang et al., 2008). The Vendor B kit uses polyclonal detection and capture antibodies raised against full-length human FGF21 (His29-Ser209), whereas the Vendor A kit uses a monoclonal capture antibody and polyclonal detection antibodies directed against the same sequence. It was recently shown that FGF21 is proteolytically processed in human plasma (Coppage et al., 2016; Dunshee et al., 2016; Zhen et al., 2016), hence we hypothesized that these kits could detect different FGF21 fragments. Consistent with this possibility, there was poor intraindividual correlation in FGF21 levels measured by the different kits ($r^2 = 0.00047$, data not shown). Because the epitope recognized by the monoclonal capture antibody from Vendor A has not been mapped, we used the kit from Vendor B for subsequent analyses to mitigate the risk of failing to detect some FGF21 due to cleavage in the capture epitope targeted by Vendor A. Accordingly, plasma FGF21 levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (BioVendor, Brno, Czech Republic). Samples were stored and analyzed at Metabolic Imaging and Liver Metabolism, University of Copenhagen, Denmark. Plasma erythrocytes, hemoglobin, leukocytes, and platelets were measured by particle counts using Sysmex XN. Plasma urea, plasma creatinine, and plasma glucose were measured by enzymatic determination – absorption photometry using Cobas 8000, c702 module. Plasma sodium and plasma potassium was measured by ion-selective electrode Measurement – potentiometry using Radiometer ABL 837. Plasma C-reactive protein was analyzed by latex particle-based immunoassay (LIA) – turbidimetry using Cobas 8000, c702 modul. Plasma ALAT, ASAT, and plasma alkaline phosphatase was analyzed by enzymatic determination – absorption photometry using Cobas 8000, c702 modul. A blood-sample for pregnancy test was drawn in all women in the study and analyzed by Sandwich Elektrochemiluminescens Immunoassay (ECLIA) - photon counting using Modular E-module. Glycosylated hemoglobin (HbA1c) was analyzed by liquid chromatography (LC) – absorption photometry using Tosoh G7, Tosoh G8. Plasma thyrotropin (TSH) was analyzed by Sandwich Electrochemiluminescence-Immunoassay (ECLIA) - photon counts using Cobas 8000, e602 module. Plasma cholesterol, HDL, LDL, and triglycerides were analyzed by enzymatic determination – absorption photometry using Cobas 8000, c702 module. Free fatty acids were quantitatively analyzed by a UHPLC 1290 Infinity system (Agilent) coupled to a 6400 Triple Quad mass spectrometry system (Agilent).

**QUANTIFICATION AND STATISTICAL ANALYSES**

**Statistical analyses of Inter99 study data**

All analyses were performed in R version 3.1.1 (http://www.r-project.org), and p values below 0.05 were considered statistically significant. General linear models, adjusted for age and gender, were used to test for differences in dietary outcomes among FGF21 genotypes. Multinomial logistic regression, adjusted for age and gender, was used to test the effect of the FGF21 SNPs on the odds of being in the 2nd and 3rd tertile of sweet snacking, salty snacking, alcohol intake, and coffee consumption. Information for the 1st tertile was used as a reference. The odds of being an occasional or daily smoker were determined using never smoker as a reference. For correction of independent multiple hypothesis testing, the Benjamini-Hochberg false discovery rate method was used.

**Statistical analysis for the clinical study**

All statistical analyses were performed using the Statistical Package for SAS 9.1 (SAS Institute, Cary NC, USA) and GraphPad Prism v6.0 (GraphPad Software, La Jolla, CA). Data are presented as mean and standard deviations for normally distributed variables and Student’s t test for comparison between groups. Median and interquartile range are presented for non-normally distributed variables including FGF21, and non-parametric Wilcoxon Rank Test was used for comparison between groups. To test for the effect of time and difference between the groups in the 5 hr sucrose challenge test, a linear mixed model test was applied. Wilcoxon Rank Test was
used to compare groups at each time point relative to baseline. Incremental area under the curve (iAUC) for FGF21 was calculated as previously described (Wolever, 2004) and compared between groups by an unpaired t test. Sample size and significance level for each analysis are stated in figure legends. P-values below 0.05 were considered statistically significant.

**ADDITIONAL RESOURCES**

More information regarding the Inter99 study may be found at https://clinicaltrials.gov/ct2/show/NCT00289237.