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Chronic high-sucrose diet increases fibroblast growth factor 21 production and energy expenditure in mice☆

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Abstract

Excess carbohydrate intake causes obesity in humans. On the other hand, acute administration of fructose, glucose or sucrose in experimental animals has been shown to increase the plasma concentration of anti-obesity hormones such as glucagon-like peptide 1 (GLP-1) and Fibroblast growth factor 21 (FGF21), which contribute to reducing body weight. However, the secretion and action of GLP-1 and FGF21 in mice chronically fed a high-sucrose diet has not been investigated. To address the role of anti-obesity hormones in response to increased sucrose intake, we analyzed mice fed a high-sucrose diet, a high-starch diet or a normal diet for 15 weeks. Mice fed a high-sucrose diet showed resistance to body weight gain, in comparison with mice fed a high-starch diet or control diet, due to increased energy expenditure. Plasma FGF21 levels were highest among the three groups in mice fed a high-sucrose diet, whereas no significant difference in GLP-1 levels was observed. Expression levels of uncoupling protein 1 (UCP-1), FGF receptor 1c (FGFR1c) and β-klotho (KLB) mRNA in brown adipose tissue were significantly increased in high sucrose-fed mice, suggesting increases in FGF21 sensitivity and energy expenditure. Expression of carbohydrate responsive element binding protein (ChREBP) mRNA in liver and brown adipose tissue was also increased in high sucrose-fed mice. These results indicate that FGF21 production in liver and brown adipose tissue is increased in high-sucrose diet and participates in resistance to weight gain.

Keywords: Sucrose diet; FGF21; GLP-1; ChREBP; Energy expenditure

1. Introduction

Increase in food consumption, especially carbohydrate and fats with or without exercise insufficiency, is considered to underlie the increasing numbers of the obese population. Although it is known that a low-carbohydrate diet leads to greater weight loss than a low-fat diet in human [1,2], the effects of various compositions of low-carbohydrate diet have not been examined in detail. Sucrose is a disaccharide consisting of glucose and fructose. Rats drinking 32% sucrose solution develop obesity due to excessive caloric intake [3,4]. On the other hand, we previously found that mice fed a solid diet containing 38.5% sucrose for 5 weeks did not develop obesity [5]. It has been reported that sucrose stimulates the sympathetic nervous system and leads to an increase in the activity and/or size of brown adipose tissues (BAT) [3,6,7]. However, the mechanism by which sucrose affects BAT activity is unclear, and the long-term effects of a high sucrose-diet on BAT, which plays an important role in energy expenditure, is not known well. In addition, the involvement of humoral factors that increase energy expenditure and prevent obesity such as glucagon-like peptide 1 (GLP-1) and Fibroblast growth factor 21 (FGF21) in mice chronically fed high sucrose-diets has not been addressed.

GLP-1 is the hormone secreted from entero-endocrine L-cells upon ingestion of nutrients such as glucose and fructose, and improves glucose metabolism by potentiating glucose-induced insulin secretion from pancreatic β-cells and by suppressing glucagon secretion from pancreatic α-cells [8,9]. As an extra-pancreatic action, GLP-1

Abbreviations: BAT, brown adipose tissue; WAT, white adipose tissue; ChREBP, carbohydrate responsive element binding protein; Cidea, cell death-inducing DNA fragmentation factor alpha-like effector A; DIO, diet-induced obese; DIO2, type 2 diodinase; FGF21, fibroblast growth factor 21; FGFR1, FGF receptor 1; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GLUT1, glucose transporter 1; ITT, insulin tolerance test; KLB, β-klotho; NC, normal chow diet; PPARα, peroxisome proliferator activated receptor-α; ST, high-starch diet; SUC, high-sucrose diet; UCP-1, uncoupling protein 1; 2DG, 2-deoxyglucose.

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participates in resistance to body weight gain by slowing gastric emptying, suppressing appetite and increasing energy expenditure [8,10,11].

FGF21, a member of the fibroblast growth factor family, acts as an endocrine hormone by binding to the beta klotho-FGF receptor complex [12–14]. Liver is considered the major source of plasma FGF21, although FGF21 is expressed in various organs including pancreas, brown adipose tissue (BAT), white adipose tissue (WAT) and muscle [15–17]. WAT and BAT are considered the major target organs of FGF21, and both beta klotho and FGF receptor 1 (FGFR1) are expressed in adipocytes [14,18,19]. Brown adipocytes express uncoupling protein 1 (UCP-1) and produce heat by uncoupling oxidative phosphorylation in the inner mitochondrial membrane. The beneficial effects of FGF21 on resistance to diet-induced body weight gain have been reported by using recombinant FGF21 or transgenic mice overexpressing FGF21 [18–20]. In these studies, FGF21 increased energy expenditure and UCP-1 expression in adipose tissues (BAT and WAT), and these effects were abolished in FGFR1 deficient mice [21].

It has been reported recently that ingestion of fructose increases plasma FGF21 levels in human [22] and that 10% sucrose in drinking water increases plasma FGF21 levels in mice within 24 h [23]. The present study characterizes effect of a high-sucrose diet on FGF21 production and related metabolic parameters. To compare the effects of sucrose on FGF21 production, mice were fed a high sucrose or a high-starch diet containing identical amounts of protein and lipid.

2. Materials and methods

2.1. Animals and diets

Eight-week-old male C57BL/6 J mice were obtained from CLEA Japan (Osaka, Japan), and housed in a room under standard 12-h light/dark cycle. The room temperature was maintained between 22°C and 24°C. All animal experiments were carried out according to a protocol approved by the Kyoto and Nagoya University Institutional Animal Care and Use Committee. Mice were divided into three groups fed a normal chow diet (NC) (CLEA Japan, Osaka, Japan), a high-starch diet (ST) or a high-sucrose diet (SUC) containing 38.5% sucrose (Table 1). Body weight was measured for 15 weeks and food intake was measured after 15 weeks of feeding.

2.2. Plasma biochemical analyses

Blood glucose levels were measured with ANTSENSE DUO (Horiba, Kyoto, Japan). Plasma insulin levels were measured using Mouse Insulin ELISA Kit (Morinaga, Kanagawa, Japan). Plasma triglycerides and free fatty acid levels were determined using the Triglyceride E test and NEFA C test (Wako Pure Chemical, Osaka, Japan). Plasma leptin levels were determined using mouse/rat leptin immunoassay kit (R&D Systems, Minneapolis, MN, USA) and plasma FGF21 concentrations were determined by mouse/rat FGF21 ELISA (Biovender, Brno, Czech Republic). Plasma glucagon was measured by glucagon ELISA (Mercodia, Uppsala, Sweden). Plasma glucagon-like peptide-1 (GLP-1) was measured by GLP-1 Total ELISA (Merck Millipore, Billerica, MA, USA).

2.3. Insulin tolerance test

Insulin tolerance test was carried out after 15 weeks of feeding with NC, ST, or SUC. Mice were deprived of food for 6 h before the test. Insulin was injected intraperitoneally at a dose of 0.6 U/kg. Blood glucose levels were measured 0, 30, 60, 90, and 120 min after insulin injection.

Table 1
Composition of experimental diets

	NC	ST	SUC
Fat	12.6	12.6	12.6
Protein	29.2	13.1	13.1
Carbohydrates	58.2	74.3	74.3
(Sucrose)	-	-	(38.5)

Data are expressed as percentage of total energy. NC, normal chow diet; ST, high-starch diet; SUC, high-sucrose diet.

2.4. 2-deoxyglucose and glucose administration experiment

Blood samples were collected before and 15 min after 2-deoxyglucose (2DG) intraperitoneal administration and 15 min after oral administration of glucose (6 g/kg) for glucagon and GLP-1 assay. Oral glucose tolerance test (2 g/kg glucose) was performed as previously reported [5].

2.5. Energy expenditure

Energy expenditure was measured using CLAMS (comprehensive laboratory animal monitoring system, Columbus Instruments, Columbus, OH, USA) and ARCO-2000 Mass Spectrometer (ARCOSYSTEM, Chiba, Japan).

2.6. Isolation of tissue RNA and quantitative real-time RT-PCR

Mice were sacrificed in the fed state. Liver, BAT and WAT were collected. Total RNA was extracted from BAT, WAT, and liver using RNAiso Plus (Takara Bio, Shiga, Japan), as previously reported (5). One microgram of total RNA was reverse transcribed using the ReverTra Ace qPCR kit (Toyobo, Osaka, Japan). After cDNA synthesis, quantitative real-time PCR was carried out in 25 μl reaction containing THUNDERBIRD qPCR Mix (Toyobo) using Mx3000 qPCR system (Stratagene, La Jolla, CA, USA). The primer sequences are shown in Supplementary Table 1. The mRNA levels were normalized by those of β-actin mRNA.

2.7. Morphological analyses

Interscapular brown adipose tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 4.0 μm. The sections were deparaffinized with xylene and ethanol, and stained with hematoxylin and eosin.

2.8. Statistical analysis

Data are presented as mean±S.E.M. Statistical analysis was evaluated using unpaired, two-tailed Student's t-test or one-way or two-way ANOVA. Differences between groups were considered statistically significant when $P<.05$. GraphPad Prism for Mac OS X version 6.0f (GraphPad Software, San Diego, CA, USA) was used for statistical analysis.

3. Results

3.1. Effects of starch and sucrose on body weight and metabolic parameters

ST-fed mice showed increased body weight gain compared to NC-fed mice: a significant difference in body weight was observed after 7-weeks of feeding and thereafter. On the other hand, SUC-fed mice displayed decreased body weight compared with that of NC-fed mice after 14-weeks of feeding (Fig. 1A). Insulin tolerance test showed that SUC-fed mice had improved insulin sensitivity compared with NC- or ST-fed mice (Fig. 1B). On the other hand, blood glucose levels in SUC-fed mice were significantly higher than those in mice fed NC or ST 15 min after glucose administration (Supplementary Fig. 1). Although plasma glucose fed state levels did not differ among the three groups, plasma insulin and leptin levels in ST-fed mice were higher than those in mice fed NC or SUC. Plasma triglyceride and free fatty acid (FFA) levels were lower in mice fed ST and SUC compared with NC-fed mice (Fig. 1C).

3.2. Chronic high-sucrose diet increases energy expenditure

To elucidate the mechanism involved in resistance to body weight gain in SUC-fed mice, we examined energy expenditure in SUC-fed, NC-fed and ST-fed mice after 15-weeks of feeding. In both dark and light cycles, energy expenditure in mice fed SUC was significantly increased compared with that in mice fed NC or ST (Fig. 2A). On the other hand, food intake was significantly increased in SUC-fed mice compared with NC-Fed or ST-fed mice (Fig. 2B). These results indicate that resistance to body weight gain in SUC-fed mice is due to increased energy expenditure.