Hepatokine fibroblast growth factor 21 (FGF21) is a metabolic regulator of adipose tissue browning (1) and glycolipid metabolism (2). Therefore, it seems likely that elevated levels of circulating FGF21 are associated with leanness, but in contrast, previous studies have shown that obese patients have a higher circulating FGF21 concentration (3). The increased FGF21 levels are thought to represent an FGF21-resistant state (4), and the chronically high levels of circulating FGF21 are associated with the onset of type 2 diabetes (5, 6), metabolic syndrome (6, 7), and cardiovascular diseases (8).

A fasting state is a stimulator of FGF21 in both rodents and humans (9, 10), and circulating FGF21 has a circadian rhythm (11, 12), which corresponds to a peak FGF21 level from midnight to early morning, and FGF21 concentration steadily declines after wak-

**Summary** Previous studies suggest that circulating fibroblast growth factor 21 (FGF21) levels are elevated in patients with fatty liver, while fasting-induced secretion of FGF21 is lower in obese patients. It has been reported that soy protein prevents hepatic fat accumulation and induces FGF21 secretion. The present study was designed to evaluate the response of circulating FGF21 levels to feeding and fasting in mice fed soy protein–rich diets. For this, C57BL/6J mice were distributed into control, high-fat high-sucrose (HFHS)-casein protein, HFHS-soy protein, and HFHS-β-conglycinin diet groups. Plasma samples were collected after 10 and 11 wk either in dark periods with feeding conditions or light periods under fasting conditions using a crossover design. After a 12-wk period of feeding, HFHS-induced hepatic fat accumulation was significantly reduced in the groups fed HFHS-soy protein and HFHS-β-conglycinin as compared to that in the HFHS-casein-fed group (p<0.05). Plasma FGF21 concentration was significantly higher in the dark/feeding periods in the HFHS-casein group (p<0.05), while in the HFHS-β-conglycinin group it was higher in the light/ fasting periods (p<0.05). The amount of mesenteric fat was significantly lower in the HFHS-casein and HFHS-soy protein groups (p<0.01). The fasting-induced FGF21 secretion was significantly and negatively correlated with hepatic fat content (p<0.05). The present study revealed that hepatic fat accumulation was associated with lower fasting-induced FGF21 secretion, which was regulated better by dietary intake of soy protein. These results support the preventive effects of soy protein on central obesity.

**Key Words** FGF21, β-conglycinin, fatty liver, central obesity, high-fat high-sucrose diet
ventrating hepatic fat accumulation and/or via independent effects.

We hypothesized that hepatic fat content is associated with response of circulating FGF21 levels to feeding and fasting conditions, which is expected to be modulated by dietary soy protein intake. This study was aimed at evaluating the relationship between chronically high levels of FGF21, fasting-induced FGF21 secretion, and hepatic fat accumulation in mice fed soy protein–rich diets.

**MATERIALS AND METHODS**

**Mice and diets.** The study protocol was approved by the ethics committee of Ryukoku University (No. 2017-6). Four-week-old male C57BL/6J Jms Slc mice were purchased from Shimizu Laboratory Supplies (Kyoto, Japan). The mice were housed at controlled temperature (21–25˚C) and humidity (45–65%) on a 12 h light–dark cycle, with lights on at 00:00 h. After a 1-wk acclimation period, the mice were distributed into four groups (n=8 per group): control, high-fat high-sucrose (HFHS)-casein protein (HFHS-casein), HFHS-soy protein (HFHS-soy), and HFHS-β-conglycinin diet groups. Casein (Lactic Casein 720; Fonterra, New Zealand), soy protein (Supro 661; Solae, USA), and β-conglycinin (Lipoff; Fuji oil, Japan) were provided as dietary protein sources. It has been reported that fructose ingestion stimulates circulating FGF21 levels (23), suggesting that habitual fructose consumption might be associated with chronically high levels of circulating FGF21. Therefore, the experimental diets included higher sucrose amounts (40% of calories from sucrose) to examine the relation between elevated FGF21 levels and fasting-induced FGF21 secretion. The control group was fed only a low-fat diet (70% of calories from carbohydrates, 10% from fat, and 20% from protein). To examine the preventive effects of soy proteins on hepatic fat accumulation, the other three groups were fed with HFHS diets (40% of calories from sucrose, 40% from fat, and 20% from protein). Pellet diets were purchased from Research Diets (NJ, USA), and dietary composition is shown in Table 1. All the mice had free access to food and tap water during the 12 wk.

**Blood sample collection under feeding and fasting conditions.** Blood was sampled after 10 and 11 wk from lateral tail veins via heparinized glass capillaries. The sampling was performed two times at the end of the dark and light periods using a crossover design. Samples corresponding to the dark/feeding conditions were collected between 21:00 and 23:00 h, which is at the end of the dark period, and mice were fed ad libitum with each diet. On the other hand, samples during the light period were collected after 15 h fasting, which corresponds to sedentary and overnight fasting conditions as observed in a human study (12). Each type of feed was removed at 18:00 h, and blood was sampled between 9:00 and 11:00 h the next day, which is at the end of the light period. Thus, half the mice in each group were alternately subjected to fasting in either the 10th or 11th week. The glass capillaries were centrifuged at 12,000 rpm for 5 min (KUBOTA 3100; Kubota, Japan), and the plasma was frozen at −80˚C until analysis.

**Metabolic measurements.** Mice were placed in metabolic cages, and their expired gas was analyzed on a CO2 and O2 mass spectrometric analyzer (ARCO-2000;
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Liver was fixed in 10% formaldehyde (Mildform 10 N; 80˚C until experiments. Another part of the tissue was immediately frozen in liquid nitrogen and stored at −80˚C until analysis. 

Blood analysis. Levels of plasma FGF21, free fatty acids (FFA), and triglycerides (TG) were measured in dark/feeding and light/fasting samples. The plasma FGF21 concentration was determined with a commercially available ELISA kit (MF2100; R&D Systems, MN, USA). The working range of the assay was 31.3 to 2,000 pg/mL. The intra- and inter-assay coefficients of variation reported by the manufacturer were 2.4–6.2% and 6.0–7.4%. The plasma FFA and TG concentrations were analyzed by means of NEFA C-test Wako and Triglyceride E-Test Wako kits (FUJIFILM Wako), respectively. Serum total cholesterol (Total-C) was measured on a Cobas b 101 system (Roche Diagnostics, Japan). Serum enzymatic activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined with Transaminase CII Test Wako (FUJIFILM Wako).

Liver TG. Hepatic lipids were extracted according to the Folch method as previously described (28). Briefly, liver tissues were homogenized, and total lipids were extracted with a chloroform : methanol (2 : 1, v/v) mixture. After centrifugation, the lower phase was dried, and the dried lipid extracts were dissolved in isopropanol with 10% of Triton X-100. The TG concentration was measured by enzymatic methods (Triglyceride E-Test Wako kits; FUJIFILM Wako).

Real-time PCR. Total RNA was extracted from the liver using the NucleoSpin RNA kit (Macherey-Nagel, Germany) and from mesenteric fat with the RNeasy Lipid Tissue Mini Kit (QIAGEN). RNA concentration and purity were determined on a Nanodrop (Thermo Fisher Scientific, Japan). The isolated RNA samples, which had an A260/A280 ratio over 2.0, were reverse-transcribed by means of the PrimeScript RT Reagent Kit with gDNA Eraser (Takara Bio Inc., Japan). Thermal cycling was performed on a T100 Thermal Cycler (Bio-Rad, Japan). The synthesized cDNA was subjected to real-time PCR with SYBR Premix Ex Taq (Takara Bio Inc., Japan) on an ABI 7300 Real-Time PCR System (Applied Biosystems, Japan). Primer sequences were designed to amplify genes encoding FGF21, FGF21 receptors, FGF21 pathways, lipin synthase, uncoupling protein 1 (UCP1), and adipocytokines (Table S1, Supplemental Online Material). Relative mRNA expression was quantified by the ΔΔCt method, and the results were normalized to the

| Table 2. Body composition and blood metabolites after the 12-wk feeding period. |
|-----------------------------------------------|
| Control | Casein | Soy protein | β-Conglycinin |
| Body weight (g) | 30.7±0.6a | 44.1±0.7c | 41.8±1.5c | 38.2±1.4b |
| Liver (g) | 1.11±0.07a | 1.87±0.11c | 1.49±0.11b | 1.36±0.07ab |
| Epididymal fat (g) | 0.87±0.08a | 2.12±0.14b | 2.14±0.10a | 2.09±0.20b |
| Retroperitoneal fat (g) | 0.34±0.04a | 1.00±0.04c | 0.94±0.05g | 0.77±0.10h |
| Mesenteric fat (g) | 0.31±0.02a | 1.25±0.08b | 1.11±0.14h | 0.62±0.10g |
| Gastrocnemius muscle (g) | 0.168±0.005 | 0.179±0.002 | 0.177±0.005 | 0.179±0.007 |
| Total cholesterol (mg/dL) | 138.9±4.3a | 224.1±10.6b | 188.6±3.4a | 168.0±8.7a |
| ALT (IU/L) | 41.3±5.1a | 171.8±17.3b | 63.3±10.5a | 54.0±7.5a |
| AST (IU/L) | 19.6±1.7a | 79.5±10.1b | 36.7±9.1a | 36.2±3.0a |

AST: aspartate aminotransferase, ALT: alanine aminotransferase, IU: international units. Different letters indicate significant differences (p<0.05; one-way ANOVA followed by Bonferroni’s test), and equal letters indicate no statistically significant results. Data are expressed as mean±SE.
expression of cyclophilin. The results were expressed as a fold change relative to the control group.

Statistics. All statistical analyses were performed in the SPSS software, version 23.0 (SPSS Japan, Japan). The Kolmogorov–Smirnov test was performed to assess the normality of data distribution, and non-normally distributed data were log-transformed prior to analysis. Differences among the groups were assessed by one-way ANOVA with post hoc Bonferroni’s test. The paired t test was conducted to evaluate the differences in plasma FFA, TG, and FGF21 levels between dark/feeding and light/fasting conditions in each group. To examine the effects of hepatic fat accumulation on FGF21 secretion, associations between liver TG content and plasma FGF21 levels were evaluated using Spearman correlation coefficient. All measurements and calculated values are presented as the mean ± standard error (SE), and the level of statistical significance was set to p<0.05.

RESULTS
Effects of soy protein–rich diets on body composition and serum biochemical parameters in mice

Table 2 presents body and tissue weights after 12 wk on each diet. HFHS diet groups (casein, soy protein, and β-conglycinin) resulted in significantly greater body weights, epididymal fat, and retroperitoneal fat weights as compared with the control group (p<0.001). Liver and mesenteric fat weights were significantly higher in HFHS-casein and HFHS-soy protein groups than in the control group (p<0.01), but there was no significant difference in the liver and mesenteric fat weights between control and HFHS-β-conglycinin groups. Among the HFHS diet groups, the HFHS-β-conglycinin group had significantly lower body weights as compared with HFHS-casein and soy groups (p<0.001), and the HFHS-β-conglycinin group had significantly lower liver weights in comparison with the HFHS-casein group (p<0.001). There was no significant difference in epididymal fat weights among the HFHS diet groups, whereas retroperitoneal and mesenteric fat weights were significantly lower in the HFHS-β-conglycinin group than in the HFHS-casein and soy protein groups (p<0.05). Although there was no statistically significant difference in the retroperitoneal fat per body weights among the HFHS diet groups, the mesenteric fat per body weights were still significantly lower in the HFHS-β-conglycinin group than in the HFHS-casein and HFHS-soy protein groups (p<0.01). Weights of the gastrocnemius muscle did not differ among the groups.

Serum Total-C levels and AST and ALT activities were significantly higher in the HFHS-casein group than in the other three groups (p<0.05). There were no significant differences in serum Total-C, AST, and ALT results among control, HFHS-soy protein, and HFHS-β-conglycinin groups.

The Influence of soy protein–rich diets on energy expenditure and respiratory exchange ratio in mice

Average and total values of energy expenditure during the light and dark periods were measured during ad libitum feeding (Fig. 1). Energy expenditure per body weight in both periods was significantly higher in the control group than in the other groups (p<0.01). Among the HFHS-protein fed groups, average energy expenditure in the HFHS-β-conglycinin group was significantly higher during the dark period (p<0.05) and tended to be higher during the light period (p=0.08) as compared with the HFHS-casein and HFHS-soy protein groups. Total 12 h energy expenditure was significantly higher in the HFHS-β-conglycinin group during both the dark and light periods (p<0.05). There was no significant difference in energy expenditure per body weight between the HFHS-casein and soy groups. The respiratory exchange ratio was significantly higher in the control group than in the other groups (p<0.01), and was not significantly different among the mice fed HFHS-casein, soy, and β-conglycinin diets.
Effects of soy protein–rich diets on liver histology and TG content in mice

As depicted in Fig. 2, HE staining uncovered histological changes and lipid accumulation in the liver of mice fed HFHS diets as compared with the control group. The largest lipid droplets were observed in the HFHS-casein group, and smaller lipid droplets were found in the HFHS-β-conglycinin group than in the HFHS-soy protein group.

Histological results were in agreement with liver TG content, and the mice fed the control diet had significantly lower liver TG content than did the other groups (Fig. 2; p<0.05). The liver TG content in the HFHS-soy and HFHS-β-conglycinin groups was significantly lower than that in the HFHS-casein group (p<0.05). Average values of the liver TG contents were higher in the HFHS-soy group than in the HFHS-β-conglycinin group, but the difference was not significant.

The influence of soy protein–rich diets on plasma FFA, TG, and FGF21 levels in the dark/feeding and light/fasting periods

Plasma FFA levels in the dark/feeding periods were significantly higher in the HFHS-β-conglycinin group than in the control and HFHS-soy groups (Fig. 3; p<0.05). The HFHS-casein group in the dark/feeding periods had significantly higher levels of plasma FFA as compared with the HFHS-soy protein group (p<0.05). In the light/fasting periods, significantly higher plasma FFA levels were detected in the control group compared with the HFHS diet groups (p<0.01). Fasting-induced increases in plasma FFA levels were found only in the control group (paired t test, p<0.05), and the HFHS-β-conglycinin group turned out to have significantly higher feeding plasma levels of FFA in comparison with fasting FFA levels (paired t test, p<0.05) in the light/fasting periods.

Approximately twofold higher levels of plasma TG were detected in the dark/feeding periods relative to the fasting conditions in all the groups (Fig. 3; paired t test, p<0.001). Plasma TG levels were significantly higher in
the HFHS-β-conglycinin group than in the other groups in the dark/feeding periods \(p<0.05\). In the light periods under fasting conditions, only the HFHS-casein group had a significantly higher plasma TG concentration than the control group \(p<0.01\).

Plasma FGF21 concentrations were significantly higher in the HFHS-casein group than in the other groups in the dark/feeding periods (Fig. 3; \(p<0.05\)), whereas the HFHS-β-conglycinin group manifested significantly higher levels of plasma FGF21 levels in the light/fasting periods as compared with the other groups \(p<0.05\). Results of the paired \(t\) test revealed significantly higher levels of plasma FGF21 in the light/fasting periods under fasting conditions than in the dark periods under feeding conditions in the control, HFHS-soy, and HFHS-β-conglycinin groups \(p<0.05\). On the contrary, only the HFHS-casein group was found to have significantly lower FGF21 concentrations in the light/fasting periods compared with in the dark/feeding periods (paired \(t\) test, \(p<0.05\)).

Correlation analysis showed that liver TG content was tended to be positively correlated with plasma FGF21 levels in the dark/feeding periods (Rho=0.349, \(p=0.059\)) and was significantly and negatively correlated with plasma FGF21 levels in the light/fasting periods (Rho=−0.399, \(p<0.05\)).

Effects of soy protein–rich diets on the expression of FGF21 and related genes in liver and mesenteric fat tissues

All mice were dissected at the end of the dark period to the beginning of the light period. Expression of hepatic FGF21 was approximately 1.5-fold higher in the HFHS-casein group than in the control and HFHS-soy groups, but this difference did not reach statistical significance (Fig. 4). There was no significant difference in the expression of the FGF21 receptors either—including the fibroblast growth factor receptor (FGFR) 1c, FGFR4, and β-klotho genes—in the liver among all the groups. The relative expression levels of activating transcription factor \(4\) (ATF4), which is reported to act upstream of FGF21 (\(21\)), were not different among the groups. Another upstream gene, peroxisome proliferator-activated receptor (PPAR) \(\alpha\), in the liver was expressed significantly more strongly in the HFHS-soy group than in the control and HFHS-β-conglycinin groups \(p<0.05\), and PPAR\(\alpha\) expression was significantly higher in the HFHS-casein group than in the control group \(p<0.01\).

Significantly high expression of PPAR target genes, including carnitine palmitoyltransferase \(1\) (CPT1) and acyl-CoA oxidase (ACO), were observed in the HFHS-casein and HFHS-soy groups compared to that in the control and HFHS-β-conglycinin groups \(p<0.01\). Expression levels of carbohydrate-responsive element–binding protein (ChREBP) and sterol-regulatory element–binding protein \(1\c\) (SREBP\(1\c\)), which stimulate lipogenic gene expression in the liver, were significantly higher in the HFHS-casein group than in the control and HFHS-β-conglycinin groups \(p<0.05\). In addition, the HFHS-casein group had a significantly higher expression level of fatty acid synthase (FAS) compared with the HFHS-β-conglycinin group \(p<0.01\).

As illustrated in Fig. 4, mesenteric fat in the control group showed lower FGF21 expression without statisti-
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In obese mice fed the HFHS-casein diet, and this result is significant lower in the control group than in the HFHS diet groups (p<0.05). There were no significant differences in the expression of FGF21 pathway–related genes (such as β-klotho and PPARγ2), ChREBP, and SREBP1c in mesenteric fat among all the groups. Although mRNA expression of UCP1 in mesenteric fat was higher in the HFHS-β-conglycinin group than in the HFHS-casein and HFHS-soy protein groups, this difference did not reach statistical significance. Expression of adiponectin in mesenteric fat tended to be higher in the HFHS diet groups than in the control group (p<0.10). In addition, leptin expression was significantly lower in the control group and significantly higher in the HFHS-casein group than in the other groups (p<0.01).

DISCUSSION

Obese individuals manifest endocrine resistance, which is characterized by an abnormally high concentration of a circulating hormone, and endocrine resistance is an independent risk factor of some diseases, as is the case for insulin resistance (29). Chronically elevated levels of circulating FGF21 are a predictive factor of metabolic diseases (5, 6, 8), and FGF21 resistance is observed in obese individuals (3) who have an attenuated fasting-induced increase in circulating FGF21 levels (12). In the present study, mice with diet-induced fatty liver showed increased and reduced plasma FGF21 levels during the dark/feeding and light/fasting periods, respectively, whereas the abnormal FGF21 secretion was prevented in non-fatty-liver mice fed soy protein–rich diets. Because it has been reported that nonalcoholic fatty liver disease is a risk factor for type 2 diabetes mellitus (30) and cardiovascular disease (31), an abnormal FGF21 secretion in patients with fatty liver may be associated with the onset of metabolic diseases.

On the other hand, injection of FGF21 itself or a variant of FGF21 improves glucose homeostasis and alleviates dyslipidemia regardless of obesity in mice and humans (13–15), suggesting that the beneficial effects of FGF21 are preserved even in an obesity-related FGF21-resistant state. In the present study, an apparent fasting-induced FGF21 secretion and lower visceral fat mass were observed in mice fed a β-conglycinin–rich diet. These results indicate the necessity to distinguish between the effects of chronically high levels of circulating FGF21 and those of temporary increases. It is possible that the decreased dark/feeding and increased light/fasting levels of circulating FGF21 denote FGF21 sensitivity. Thus, further research is needed to confirm the relation between the reactivity of FGF21 secretion and FGF21 function, including adipose tissue thermogenesis and glucolipid metabolism.

In this study, hepatic fat accumulation was prevented by both soy protein and β-conglycinin consumption in mice fed HFHS diets. The plasma FGF21 concentration in the dark periods was significantly higher in the HFHS-casein group, which had the highest hepatic TG content. Hepatic FGF21 expression tended to be higher in obese mice fed the HFHS-casein diet, and this result is in line with the elevated FGF21 concentrations in obese mice and human subjects (3, 4). The HFHS-soy protein group turned out to have lower plasma FGF21 levels under the dark/feeding conditions as compared with the HFHS-casein group. Correlation analysis hinted that liver TG contents may be associated with basal plasma FGF21 levels in the dark/feeding periods, though not to a statistically significant level. Our data support previous reports (16–18) suggesting that hepatic fat content was associated with high levels of plasma FGF21 levels under the dark/feeding conditions.

PPARs are ligand-activated transcription factors, and FGF21 is induced by activation of PPARα in the liver and of PPARγ in adipose tissue (32). Besides, fasting-induced increases in FFA levels facilitate expression of the hepatic PPAR–FGF21 pathway (33). In the present study, the control group showed higher plasma FFA and FGF21 concentrations in the light periods under the fasting conditions than in the dark periods under the feeding conditions, whereas the fasting response of FFA was not in agreement with that of the HFHS diet groups. Circulating FGF21 levels were increased by fasting for 15 h in the control, HFHS-soy, and HFHS-β-conglycinin groups as described elsewhere (34), whereas only the HFHS-casein group yielded the results contradicting the fasting-induced increase in circulating FGF21. Our correlation analysis revealed that liver TG contents were associated with fasting-induced FGF21 secretion, suggesting that hepatic fat content is associated with FFA-independent effects on FGF21 secretion in mice fed HFHS diets. These findings support the claim that soy protein consumption prevents the abnormal secretion of FGF21 that is induced by the HFHS diet. In addition, chronically high levels of FGF21 are likely to cause compensatory dysfunction of hepatic FGF21 secretion, similar to pancreatic β cell failure in insulin-resistant hyperglycemia (29). High levels of plasma FGF21 levels in the dark/feeding periods may similarly lead to lower levels of fasting-induced FGF21 secretion seen in the present study.

The highest average concentration of plasma FGF21 was observed in mice fed the HFHS-β-conglycinin diet in the light periods under fasting conditions. A previous study reported that hepatic FGF21 expression is increased by the ATF4-FGF21 axis in mice fed β-conglycinin diets (21). Although the hepatic ATF4 expression was measured in the present study, the real-time PCR analysis was not performed under the fasting condition. It is possible that ATF4 regulates the marked increase in fasting FGF21 levels. Further experiments are needed to investigate the association between the ATF4-FGF21 axis and the response of FGF21 expression to both feeding and fasting conditions. In addition, the mice fed the HFHS-β-conglycinin showed lower mesenteric fat weight, which is representative of visceral fat mass. The preventive effects of β-conglycinin on visceral fat accumulation are consistent with another interventional study (35), and our cross-sectional study has revealed an association between higher serum FGF21 levels and a smaller visceral fat area (36). Here, epididymal fat...
weight did not differ among the HFHS diet groups, and thus the effect of dietary β-conglycinin may be specific to visceral adipose tissue. These results suggest that FGF21 modulates body fat distribution. Because the mice fed the HFHS-β-conglycinin showed the chronically high levels of FGF21 and the apparent fasting-induced FGF21 secretion, the sensitivity and reactivity of FGF21 may exert preventive action on visceral fat accumulation. Blood flows into the liver via the portal vein, which means that visceral fat is the most distant tissue to respond to liver-derived FGF21. It is therefore possible that FGF21 actions are influenced by the location of the adipose tissues.

This study uncovered an approximately twofold higher plasma FGF21 concentration in the dark periods under feeding conditions as compared with other studies (4, 21). The experimental diets included fructose, which stimulates FGF21 secretion (23), and thus habitual fructose consumption might be associated with chronically high levels of circulating FGF21. Furthermore, a recent study indicates that ChREBP has an important role in FGF21 secretion after fructose intake (37). This finding is in agreement with our data showing that mice fed the HFHS-casein diet had higher expression of ChREBP and plasma FGF21 levels during the dark periods. It is considered that the chronic expression of ChREBP is related to an increase in basal secretion of FGF21, and this may be reason for the lower fasting plasma FGF21 levels in the HFHS-casein group, but not in the other groups. On the other hand, the lean mice fed the control diet showed FGF21 levels in the dark periods that were similar to those of the mice fed the HFHS diets rich in soy proteins, regardless of different liver TG content. Although further study is needed to explore the precise mechanism, there was no significant difference in the expression levels of hepatic ChREBP among the control, HFHS-soy protein, and HFHS-β-conglycinin fed groups. The results suggest the possibility that fructose-induced ChREBP expression is associated with circulating FGF21 levels in the dark/feeding condition in the present study. Furthermore, one study has revealed that FGF21-deficient mice fed a control diet undergo a mild weight gain but manifest a marked body weight gain and body fat accumulation when fed a ketogenic (high-fat) diet (38). These observations suggest that a lack of FGF21 is related to an impaired ability to metabolize lipids on a high-fat diet; therefore, mice fed a control low-fat diet had a lean phenotype in this study despite the high levels of plasma FGF21.

This study evaluated gene expression in the liver and visceral adipose tissue, which underwent smaller accumulation in the mice fed the HFHS-β-conglycinin diet. FGFR1 is the major receptor for FGF21 (39), and FGF21 signaling requires both FGFR and β-klotho, which form the FGF21–FGFR–β-klotho complex (40). In the present study, there were no significant differences in FGFR4 and β-klotho expression levels among the groups. Hepatic FGFR1c was not significantly different among the groups, whereas FGFR1c was highly expressed in mesenteric fat tissue of the mice fed HFHS diets. Although the underlying mechanism is unclear, another study also indicates that FGFR1 expression is high in subcutaneous adipose tissues of obese humans and rats (41). These results suggest that FGFR1 is an obesity-induced gene in both subcutaneous and visceral adipose tissues, and this induction may be partly caused by overnutrition.

PPARγ2 expression in mesenteric fat did not differ among the groups, whereas the hepatic PPARα gene was upregulated in the HFHS-casein and HFHS-soy protein groups but not in the HFHS-β-conglycinin group. CPT1 and ACO, which are PPAR target genes, showed similar expression patterns as PPARα in the liver, suggesting that the HFHS-diet induced the PPAR pathway in the HFHS-casein and HFHS-soy protein groups. It has been reported that PPARα is induced by isoflavone, which is a component of soy and participates in the reduction in hepatic fat contents (42). Therefore, hepatic fat accumulation may be inhibited by PPARα induction in the HFHS-soy protein group. Hepatic PPARα expression was significantly higher in the HFHS-casein group too, but there were significantly higher mRNA levels of FAS, ChREBP, and SREBP1c (which are lipogenesis genes) in the HFHS-casein group. These data suggest that these lipogenesis genes are more strongly associated with hepatic fat accumulation than PPARα expression. Moreover, the present study indicates that β-conglycinin intake suppresses hepatic FAS, ChREBP, and SREBP1c expression. This result suggests that dietary β-conglycinin prevents expression of these lipogenesis genes, thereby preventing hepatic fat accumulation, regardless of PPARα expression status.

Some studies have revealed that FGF21 stimulates browning of adipose tissues, and this process upregulates UCP1, thereby increasing energy expenditure and reducing body fat content (43). The expression of thermogenic protein UCP1 was approximately 1.5-fold higher in the HFHS-β-conglycinin group, but this difference did not reach statistical significance. Some studies have suggested that UCP1 has a circadian expression pattern in brown adipose tissue (44), and therefore it is possible that the sampling times influenced the FGF21-induced changes in UCP1 expression in adipose tissue. In addition, our metabolic measurements revealed a higher energy expenditure in the HFHS-β-conglycinin group. It is well known that fat-free mass is a strong predictor of energy expenditure (45), and intraperitoneal fat was smaller in the mice fed the HFHS-β-conglycinin diet than in the other HFHS diet groups. This result suggests that fat-free mass per body weight may be higher in the HFHS-β-conglycinin group than in the other HFHS diet groups, and thus consideration needs to be applied to the factors of higher energy expenditure in mice fed the β-conglycinin diet.

Adipose tissue is an endocrine organ that performs a critical function in metabolic homeostasis. It is believed that adiponectin has beneficial effects on glycolipid metabolism and is negatively associated with visceral fat mass (46); however, the present study showed that adiponectin expression was approximately twofold higher
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in the HFHS diet groups than in the control group. It has been reported that PPARγ has a role in transcriptional activation of adiponectin (47), and the expression levels of PPARγ2 and adiponectin manifested similar patterns in the present study. Therefore, it is likely that adiponectin expression was influenced by PPARγ, regardless of visceral fat mass.

Leptin is another adipocytokine that regulates food intake and energy homeostasis, and obese individuals show leptin resistance (48). It has been reported that a high-fat high-fructose diet increases both leptin expression in adipose tissue and circulating leptin levels (49), and the present study revealed that leptin expression in mesenteric fat was elevated in the HFHS-casein diet group but not in the HFHS-soy protein and HFHS-β-conglycinin groups. This result suggests that dietary soy proteins suppress HFHS diet-induced leptin resistance. Although further analysis is needed regarding the relation between leptin resistance and fatty liver (50), it has been demonstrated that serum leptin levels are elevated in patients with fatty liver, independently of the body-mass index and percentage of body fat (51), and leptin improves fatty liver by stimulation of β-oxidation (52). It was reported that leptin suppressed the lipogenic enzyme gene by down-regulating the SREBP1c gene (53), suggesting that in the HFHS-β-conglycinin group the lower leptin resistance was associated with lower expression levels of FAS and ChREBP, which was regulated by SREBP1c. The leptin-regulated SREBP1c expression may be associated with plasma FGF21 levels, which is induced by ChREBP as seen in the HFHS-casein group. These observations point to the preventive effects of leptin on hepatic fat accumulation, and suppressed leptin expression may be associated with lower hepatic fat content and FGF21 secretion in the HFHS-soy protein and HFHS-β-conglycinin groups under feeding conditions.

This study has several limitations mainly due to time points for the sampling of blood and tissues. Chronological observation makes it possible to precisely determine the effects of FGF21 resistance on a body fat distribution; however, it is difficult to collect blood and tissue samples at all time points, and a large number of animals would be necessary. Noninvasive techniques, such as luciferase bioluminescence imaging, are necessary in future studies, at least for gene expression measurements.

This study reveals that hepatic fat accumulation is associated with an abnormal FGF21 secretion, which is prevented by soy protein–rich diets. Mice fed HFHS-soy protein and HFHS-β-conglycinin diets showed lower hepatic fat content, which is associated with lower plasma FGF21 levels in the dark/feeding periods and higher fasting-induced plasma FGF21 levels. These results suggest that hepatic fat content is a determinant of chronically high levels of circulating FGF21 and the attenuated FGF21 secretion under fasting conditions. In addition, mice fed the HFHS-β-conglycinin diet had a marked increase in plasma FGF21 levels under fasting conditions, as well as lower visceral fat mass. These results support the role of hepatic fat content in FGF21 metabolism and the beneficial effects of soy protein intake on body fat distribution.

Disclosure of state of COI

No conflicts of interest to be declared.

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Supporting information

Supplemental online material is available on J-STAGE.

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