Glycative Stress Research

Online edition: ISSN 2188-3610
Print edition: ISSN 2188-3602
Received: May 18, 2019
Accepted: July 9, 2019
Published online: December 31, 2019
doi:10.24659/gsr.6.4_198

Original article

Anti-obesity effect of Echigoshirayukidake (*Basidiomycetes-X*) in rats

Shinji Sato [1)], Misato Sakurai [1)], Tetsuya Konishi [1)], Koji Nishikawa [2)], Yoshiaki Tsuno [2)]

1) Faculty of Applied Life Sciences, Niigata University of Pharmacy and Applied Life Sciences, Niigata, Japan
2) Mycology Techno Co., Ltd, Niigata, Japan

Abstract

***Purpose*:** The edible mushroom Echigoshirayukidake (*Basidiomycetes-X*: BX) is rich in β-glucan, and BX extracts have antioxidant and immunostimulatory effects. This study examined the preventive effect of BX on the onset of metabolic syndrome in rats fed a High-Fat High-Sugar (HFHS) diet supplemented with BX.

***Methods*:** In Experiment 1, male Wistar rats were divided into 3 groups: Control (reared on a standard diet), HFHS (HFHS diet) and HFHS+BX (HFHS feed supplemented with 5% BX dry powder). In Experiment 2, rats were divided into 5 groups: Control, HFHS, HFHS+BX-T (5% BX dry powder), HFHS+BX-E (5% BX water extract) and HFHS+BX-R (5% BX extract residue) (n=9–10 each). After 15 weeks on the respective diets, visceral fat, liver tissue and biochemical parameters in blood were examined. A glucose tolerance test (GTT) was also performed.

***Results*:** The HFHS group showed body weight gain; visceral fat accumulation; elevated blood AST, ALT and LDH levels; and histological findings of fatty liver. The HFHS+BX group showed suppressed body weight gain; visceral fat accumulation; decreased blood AST, ALT, LDH, TC and LDL-C levels; and mild fatty liver. There was also a drop in blood glucose levels in the HFHS+BX-T group 15 to 20 minutes following GTT. The improvement in visceral fat accumulation was most notable in the HFHS+BX-E group among the BX-supplemented groups.

***Conclusion*:** Intake of BX-E extract has preventive effects on visceral fat accumulation (metabolic syndrome) and fatty liver induced by an HFHS diet, suggesting that BX-E extract may be a useful functional food component.

***KEY WORDS*:** Echigoshirayukidake (*Basidiomycetes-X*), metabolic syndrome, fatty liver, visceral fat, insulin resistance

Introduction

Metabolic syndrome causes postprandial hyperglycemia due to increased insulin resistance. This pathological condition is also thought to increase the risk of cerebrocardiovascular events. The increasing morbidity due to this condition in recent years has focused attention on a number of functional foods. Among mushrooms, the shiitake mushroom (*Lentinula edodes*) contains a functional component called eritadenine, which has cholesterol-lowering effects. Kawariharatake (*Agaricus subrufescens*) is rich in γ-aminobutyric acid (GABA), which is thought to improve hypertension. Echigoshirayukidake (*Basidiomycetes-X*: BX) is a newly discovered type of edible mushroom found in Uonuma, Niigata Prefecture, that is characterized by its atypical mushroom shape [1, 2]. While the carbohydrate, protein and dietary fiber content of BX is similar to that of other mushrooms, it is distinguished by its high β-glucan content. BX is known to have functional properties, including antioxidant effects characterized by high levels hydroxy radical-scavenging activity, beneficial effects on skin in patients with atopic dermatitis, and immunostimulatory effects [1, 2].

In this study, we examined the preventive effect of BX on the onset of metabolic syndrome in rats fed a High-Fat High-Sugar (HFHS) diet supplemented with BX.

Corresponding to: Shinji Sato, PhD
Faculty of Applied Life Sciences, Niigata University of Pharmacy and Applied Life Sciences
265-1 Higashijima, Akiha-ku, Niigata 956-8603, Japan
TEL: +81-250-25-5000 FAX: +81-250-25-5127 eMail: sato@nupals.ac.jp
Co-authors: Sakurai M, sakurai@nupals.ac.jp; Konishi T, t.konishi@bg.wakwak.com;
Nishikawa K, nishikawa@mycologytechno.com; Tsuno Y, tsuno@mycologytechno.com

Methods

Experiment 1

Male Wistar rats (4 weeks old) were used. The rats were divided into the following three groups: (i) Control animals were fed an AIN-93M standard diet (n = 10); (ii) HFHS (n = 10) animals were fed an HFHS diet; and (iii) HFHS+BX animals were fed HFHS feed supplemented with 5% BX dry powder (n = 9).

The flow of **Experiment 1** is shown in ***Fig. 1***. After a 2-week habituation period, each group was placed on their respective diet. The amount of food consumed was measured daily, and body weight was measured once a week for 100 days. Caloric intake in the control and HFHS+BX groups was adjusted such that consumption was equal to that of the HFHS group. Subsequently, a glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed to examine the effect of BX on insulin resistance (***Fig. 2***). Rats were intravenously administered 0.833 g/kg glucose for GTT and 0.5 IU/kg insulin for ITT, and a catheter was subsequently inserted into the jugular vein for blood collection over 4 hours. Blood plasma glucose, free fatty acid (FFA) and triglycerides (TG) were measured. After a 2-week recovery period, the rats were sacrificed and dissected. Visceral fat (mesenteric, perirenal and epididymal fat) and liver samples were weighed, and histological examination, measurement of fat content and blood tests were performed. Liver fat content, TG and total cholesterol (TC) levels were measured after tissue homogenization. Fasting plasma glucose (FPG), insulin, FFA, TG, TC, low-density lipoprotein-cholesterols (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) levels were measured from plasma.

Experiment 2

Experiment 2 was conducted to determine which fraction contained the functional components of BX, and whether there was a difference in effect by fraction (***Fig. 1***). Wistar male rats (5 weeks old) were used for these experiments. The rats were habituated for 1 week, during which time they were divided into the following 5 groups: (i) Control rats were reared on a standard diet (modified AIN 93-M) (n = 10); (ii) HFHS rats were reared on an HFHS diet (n = 10); (iii) HFHS+BX-T rats were reared on HFHS feed supplemented with BX dry powder (BX-T) (n = 10); (iv) HFHS+BX-E rats were reared on HFHS feed supplemented with BX water

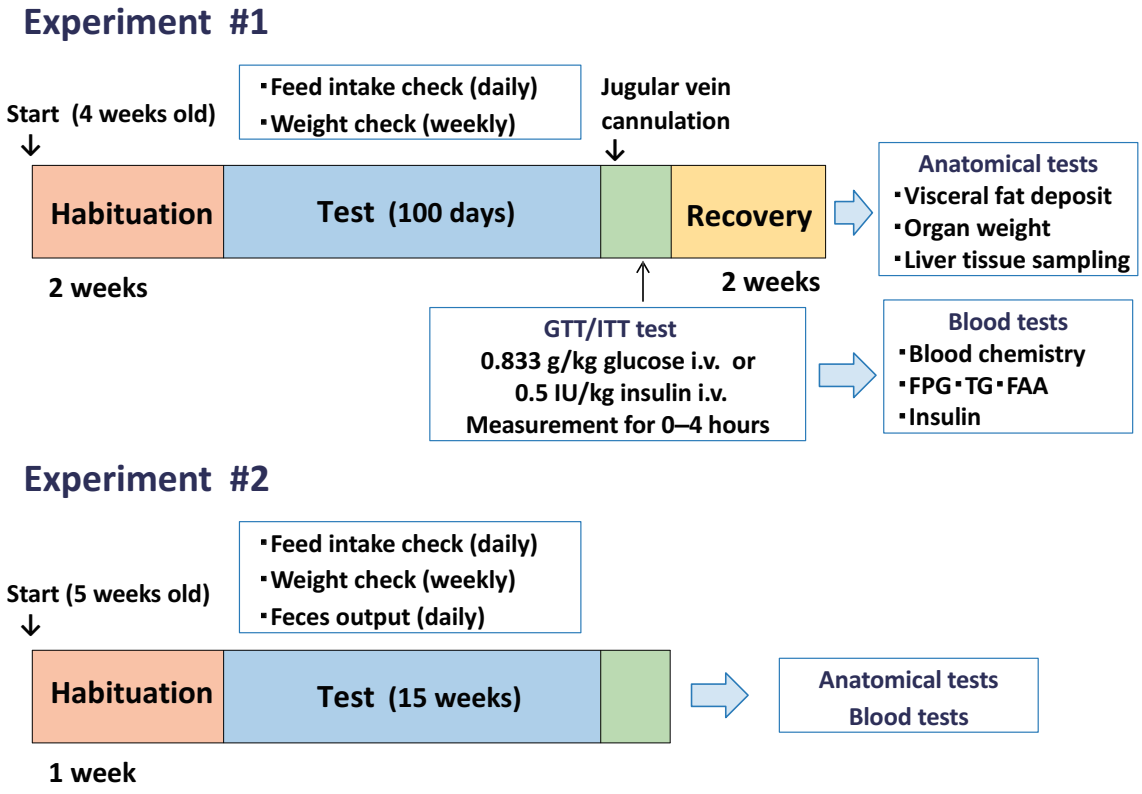

Fig. 1. **Experimental design for Experiment 1 and 2.**

GTT, glucose tolerance test; ITT, insulin tolerance test; FPG, fasting plasma glucose; TG, triglyceride; FAA, free fatty acid; i.v., intravenous.

extract dry powder (BX-E) (n = 10); and (v) HFHS+BX-R rats were reared on HFHS feed supplemented with BX water extract residue powder (BX-R) (n = 10).

Table 1 shows the component analysis results for BX-T, BX-E and BX-R. All supplements were added until they comprised 5% of the feed. The rats were fed their respective diets for 15 weeks after habituation, during which time the amount of food consumed was measured daily, body weight was measured once a week, and fecal lipid output was measured once. Fecal lipid output was measured by weighing the dry weight after collecting and drying all feces produced by each rat daily across the 15 weeks. TC and TG content in the feces was also measured. Rats were sacrificed and dissected after rearing for 15 weeks. Visceral fat weight and liver fat content were measured, and liver histological examination and biochemical examination of blood were performed as described for **Experiment 1**.

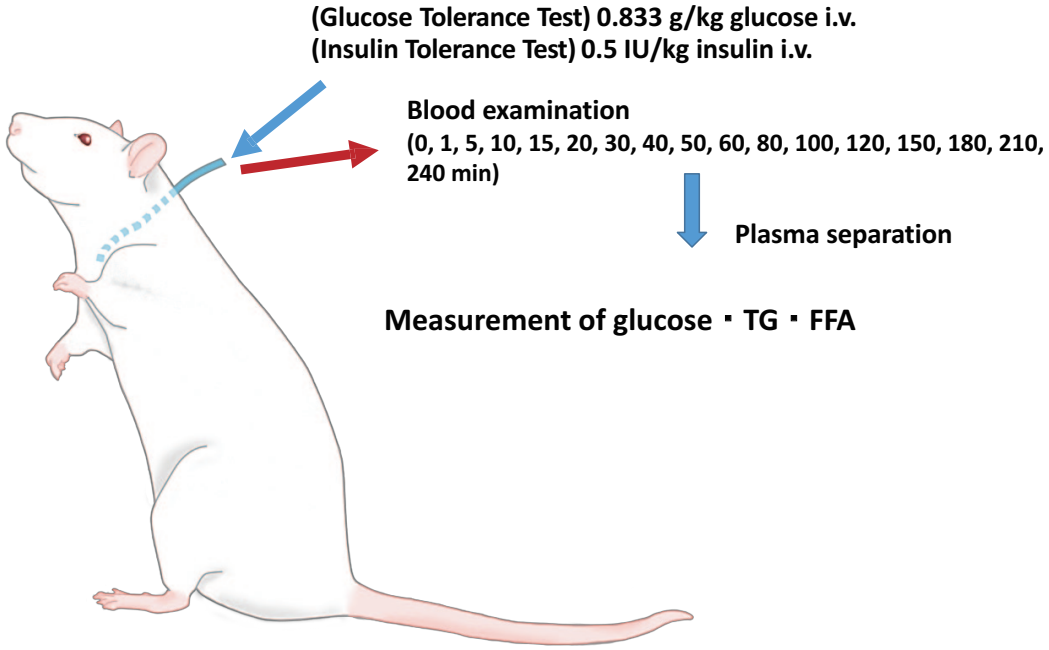

Fig. 2. **Experimental design for GTT and ITT.**

GTT, glucose tolerance test; ITT, insulin tolerance test; TG, triglyceride; FAA, free fatty acid; i.v., intravenous.

Component (per 100 g)	BX-T	BX-E	BX-R
Energy (kcal)	179	372	311
Water (g)	8.2	0.8	0.4
Protein (g)	16.0	4.7	21.0
Fat (g)	1.9	0.2	1.9
Carbohydrate (g)	36.7	86.5	30.9
Dietary fiber (g)	32.7	2.9	43.3
Ash (g)	4.5	4.9	2.5
Na (mg)	10.1	12.0	4.0

BX-T, 5% BX dry powder; BX-E, 5% BX water extract; BX-R, 5% BX extract residue; BX, *Basidiomycetes-X*.

Results

Experiment 1

Measurement of the daily and total amount of food consumed showed that the HFHS groups (HFHS and HFHS+BX groups) consumed less food than the control group (***Fig. 3***). The change in body weight gain was greater in the HFHS group than in the control group (***Fig. 4***). Comparison of HFHS and HFHS+BX groups showed that body weight gain was significantly lower in the HFHS+BX group from 11 to 20 weeks ($p<0.01$), even though the two groups consumed a similar amount of food.

Comparison of the change in visceral fat weight revealed significantly higher levels of mesenteric, perirenal and epididymal fat in the HFSH group compared to the control group. Comparison of the HFHS and HFHS+BX groups showed that fat accumulation was significantly lower in the HFHS+BX group (***Fig. 5***).

Comparison between the HFHS and HFHS+BX groups showed that liver weight and liver fat content, and total fat, TG, and TC in liver tissue were all significantly lower in the HFHS+BX group (***Fig. 6***).

Blood chemistry analysis (***Fig. 7***) demonstrated that aspartate transaminase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were significantly elevated in the HFHS group compared to the control group. In contrast, these levels were significantly lower in the HFHS+BX group than in the HFHS group, decreasing to levels similar to those in the control group. In glycolipid metabolism, there was no significant difference in FPG, insulin, FFA or TG among the 3 groups. However, comparison of the HFHS and HFHS+BX groups showed that TC and LDL-C levels in the HFHS+BX group were significantly decreased compared to those in the HFHS group. There were no significant differences in HDL-C levels.

Changes in blood glucose, TG and FFA were also measured following the GTT and ITT (***Fig. 8-10***). B lood glucose levels at 15 to 20 minutes following the GTT were significantly lower in the HFHS+BX group than the HFHS group. There were no significant differences in TG or FFA among the 3 groups. Further, there were no significant differences in post-ITT blood glucose, TG or FFA among the 3 groups.

Experiment 2

Measurement of the amount of food consumed showed that the 4 groups that received the HFHS diet consumed 15% less food than the control group (***Fig. 11***). Despite this, total caloric intake was 8,000 kcal per animal in all groups, and there were no significant differences among the 5 groups. Body weight was lower in the groups that received BX (HFHS+BX-T, HFHS+BX-E and HFHS+BX-R groups) than in the HFHS group, and body weight was significantly lower in the HFHS+BX-E group (***Fig. 12***).

The increase in visceral fat weight that occurred following consumption of the HFHS diet tended to decrease with administration of BX. In particular, perirenal fat weight was significantly lower in the HFHS+BX-E than the HFHS group (***Fig. 13***).

The results of feces lipid output measurements are shown in ***Fig.14***. There were no significant differences in the amount of feces excreted or fecal lipid output among the 5 groups. Fecal TG output was significantly higher in the HFHS+BX-R group than the HFHS group. Fecal TC output was significantly lower in the HFHS+BX-T and HFHS+BX-R groups than the HFHS group.

Histological images of the liver are shown in ***Fig. 15***. In the control group, the liver tissue comprised normal hepatocytes with clearly distinguishable hepatocyte cords and sinusoids. In the HFHS group, there was significant accumulation of fat in hepatocytes, indistinguishable hepatocyte cords and signs of fatty liver. In contrast, in the HFHS+BX-T and HFHS+BX-R groups, there was moderate accumulation of lipid droplets in hepatocytes and mild structural disturbance to hepatocyte cords. In the HFHS+BX-E group, there was significant accumulation of lipid droplets in hepatocytes, while the structure of hepatocyte cords was mostly maintained.

Total fat levels in the liver are shown in ***Fig. 16***. Total fat, TG, and TC levels in liver tissue were all significantly lower in the HFHS+BX-E group than the HFHS group.

Blood biochemistry analysis (***Fig. 17***) showed that there were no significant differences in FPG, TG, or FFA levels among the 5 groups. Further, there were no significant differences in AST or SLT between the HFHS group and BX-supplemented groups (HFHS+BX-T group, HFHS+BX-E group, HFHS+BX-R group).

Discussion

In **Experiment 1**, rats fed an HFHS diet showed body weight gain; visceral fat accumulation; increased levels of fat in liver tissue; elevated levels of AST, ALT and LDH; histological findings of fatty liver; and increased fecal TC output. Compared to the HFHS group, the group that received HFHS feed supplemented with BX extract showed suppression of body weight gain and visceral fat accumulation; a reduction in elevated levels of fat in liver tissue; lower blood AST, ALT, LDH, TC and LDL-C levels; lower lood glucose level at 15 to 20 minutes following GTT, and improved histological signs of fatty liver. These results suggest that BX has a preventive effect on the onset of HFHS-induced metabolic syndrome and fatty liver.

In **Experiment 2**, the effects of BX-T, BX-E and BX-R were compared. BX-E was observed to have the most notable effect on suppressing body weight gain and perirenal fat accumulation, reducing elevated levels of fat in liver tissue (total lipid, TC and TG), and reducing the severity of histological findings of fatty liver. These results suggest that BX-E, a dry powder form of BX extract, was most effective. Given that none of the effects of BX-E were observed with BX-R, a dry powder form of the extract residue, we speculate that the functional components of BX are present in the extract.

Although the functional components in BX are currently unknown, the various physiological effects of BX shown in this study suggest that BX contains various functional components.

(1) Effect on animal fat dependence

Recent studies have found that, due to high-fat diets, signs of endoplasmic reticulum stress can be observed in neurons in the reward system linked to hypothalamic metabolism, leading to animal fat dependence, an increase in preference for fat

intake, and consequently resulting in the neglect of exercise and an increase in fat accumulation [3, 4]. γ-Oryzanol is a known functional component of rice germ that alleviates endoplasmic reticulum stress and attenuates dependence on animal fat [3, 5]. In the present study, the HFHS+BX group showed slightly lower food intake than the HFHS group (***Fig. 3-a***). However, it was difficult to determine whether this was due to a reduction in appetite. Further studies are needed to examine whether BX extract is useful for alleviating animal fat dependence.

(2) Improvement effect on insulin resistance

Given that the HFHS+BX group showed lower blood glucose levels at 15 to 20 minutes following GTT compared to the HFHS group (***Fig. 8-a***), we predict that BX improved the effects of insulin. Because there were no significant changes in blood insulin levels or FPG, and the reduced glucose levels were observed in the early period, we speculate that the ability of pancreatic β cells to secrete insulin improved in response to hyperglycemia. Factors that affect insulin secretion include (1) involvement of incretin (increased GLP-1 and GIP secretion) [6-8], (2) involvement of dopamine type 2 receptors in pancreatic β cells [9, 10], (3) reduction in endoplasmic reticulum stress in pancreatic β cells [9, 10], and (4) involvement of short-chain fatty acid receptors (GPR41/GPR43) [11-13]. The effect of BX extract on the ability of pancreatic β cells to secrete insulin should be examined in future studies.

(3) Effect on gut microbiota

High-fat diets also have an effect on the gut microbiota [14-16]. In rats, high-fat diets have been shown to lead to an increase in lactic acid bacteria of the genus *Lactobacillus*, bacteria of the genus *Clostridium coccoides* (named *Blautia coccoides* from 2008 onward) and *Bacteroides*, a decrease in the genus *Prevotella*, and an increase in the genus *Clostridium leptum* [17]. These changes in the gut microbiota are expected to reduce the production of short-chain fatty acids in the intestinal tract. These changes are alleviated by intake of astaxanthin, an antioxidant with a low absorption rate, with 95% or more of the compound remaining in the intestinal tract to exhibit antioxidant functions. Astaxanthin may therefore also have a positive effect on gut microbiota. Similarly, while human type 2 diabetes patients show almost no change in their total enterobacteria count, they exhibit a significant increase in bacteria of the genus *Clostridium coccoides*, *Clostridium leptumgenus* and *Lactobacillus* of the phylum *Firmicutes*, and a significant decrease in the genus *Prevotella*. They also exhibit lower levels of short-chain fatty acids in feces [18]. Short-chain fatty acids bind to GPR41/GPR43 on pancreatic β cells as a ligand to promote the secretion of insulin. Additionally, short-chain fatty acid receptors mediate effects such as elevated body temperature, heart rate, and basal metabolism, which act in a concerted manner to prevent weight gain and metabolic syndrome. The effect of BX extract on gut microbiota should be examined in future studies.

(4) Effect on brown fat

Two types of thermogenic fat cells, brown and beige adipocytes, play an important role in controlling systemic energy metabolism. When a warm-blooded animal is exposed to the cold, its sympathetic nerves are activated, and brown adipocytes induce rapid thermogenesis. At the same time, the expression of genes involved in fat burning and thermogenesis is rapidly induced, maximizing the thermogenic ability. As a result, metabolic disorders such as type 2 diabetes can improve in the absence of any improvement in insulin secretion [19-22].

Recent studies have reported that factors such as a cold environment induce the production of beige adipocytes. This phenomenon is called "beiging" of white adipose tissue and is an important mechanism for adaptation to long-term exposure to cold environments [21]. Induction of the production of beige adipocytes is expected to lead to suppression of obesity and improvement in systemic glucose and lipid metabolism [22]. PRDM16, a transcription coregulator, and EHMT1, a histone modifier, play significant roles in the generation of brown and beige adipocytes [22]. In addition to thermogenesis-mediated effects, brown and beige adipocytes are thought to be directly involved in insulin resistance [23].

In the present study, visceral fat (white fat) was significantly reduced when HFHS feed was supplemented with BX extract, suggesting that the functional component in BX may have an effect on the whitening and browning of adipocytes. Whether BX extract increases levels of brown and beige adipocytes should be determined in future studies.

(5) Lipid output in feces

Lipid output in feces was examined in **Experiment 2**. We found that there were no significant differences in fecal output, TG, TC or total fat in feces between the HFHS and HFHS+BX groups (***Fig. 14***), suggesting that BX extract may not increase lipid output in feces. However, it was not possible to determine why the fecal TG output was significantly higher in the HFHS+BX-R group than the HFHS group (***Fig. 14c***), or why the fecal TC output was significantly lower in the HFHS+BX-T and HFHS+BX-R groups than the HFHS group (***Fig. 14d***). Given that BX-R does not contain BX extract, we hypothesize that these findings are not related to the functional components of BX extract.

Conclusion

Animal experiments showed that intake of the dry powder form of Echigoshirayukidake extract has a preventive effect on visceral fat accumulation (metabolic syndrome) and fatty liver induced by an HFHS diet. This study suggests that BX may be useful as a functional food for preventing metabolic syndrome and fatty liver, particularly given that humans have long consumed BX as a food product and BX has been proven to be a safe food item.

Conflict of Interest Statement

The present study was supported by Mycology Techno Co., Ltd.

Acknowledgement

Part of this study was presented at the meeting of the Society for Echigoshirayukidake on October 17, 2015 in Tokyo.

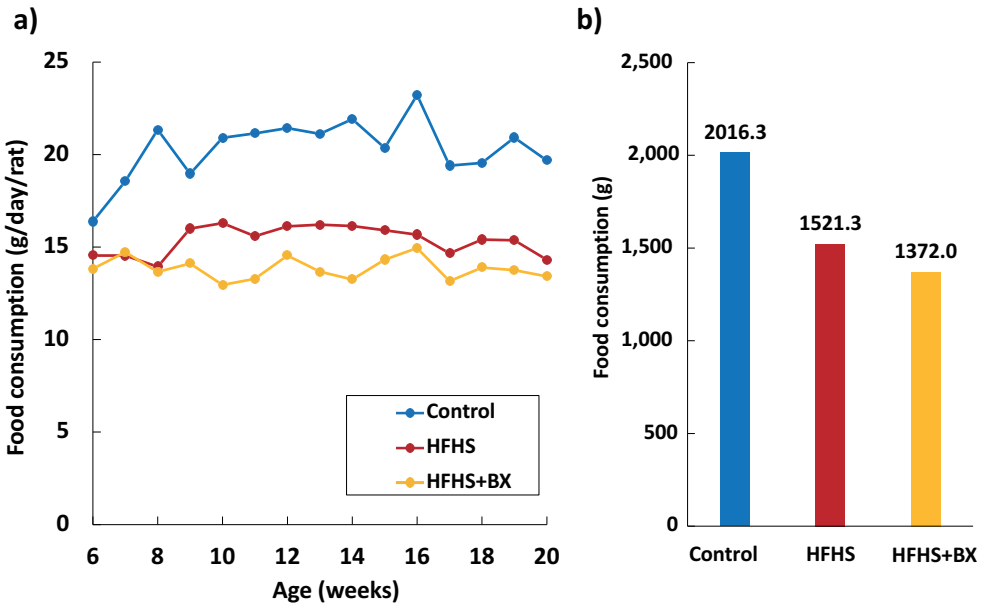

Fig. 3. **Food consumption.**

a) Daily food consumption and **b)** total food consumption throughout the experiment. Control, n = 10; HFHS, high-fat high-sugar, n = 10; HFHS+BX, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 9; BX, *Basidiomycetes-X*.

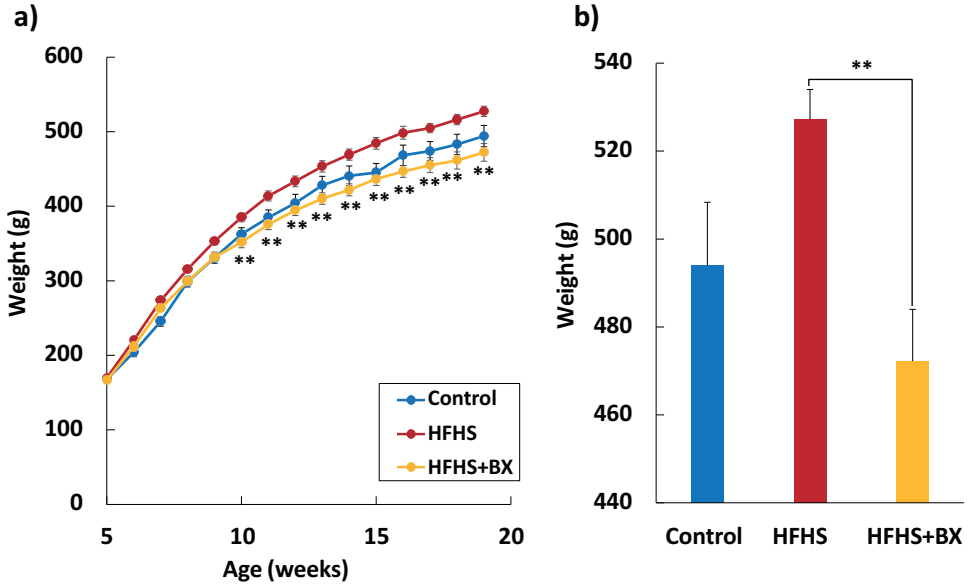

Fig. 4. **Body weight.**

a) Change in body weight. Results are expressed as mean ± SEM. **b)** Body weight at the end of the experiment. Results are expressed as mean ± SD; **p < 0.01 by the Tukey-Kramer test. Control, n = 10; HFHS, high-fat high-sugar, n = 10; HFHS+BX, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 9; BX, *Basidiomycetes-X*; SD, standard deviation; SEM, standard error mean.

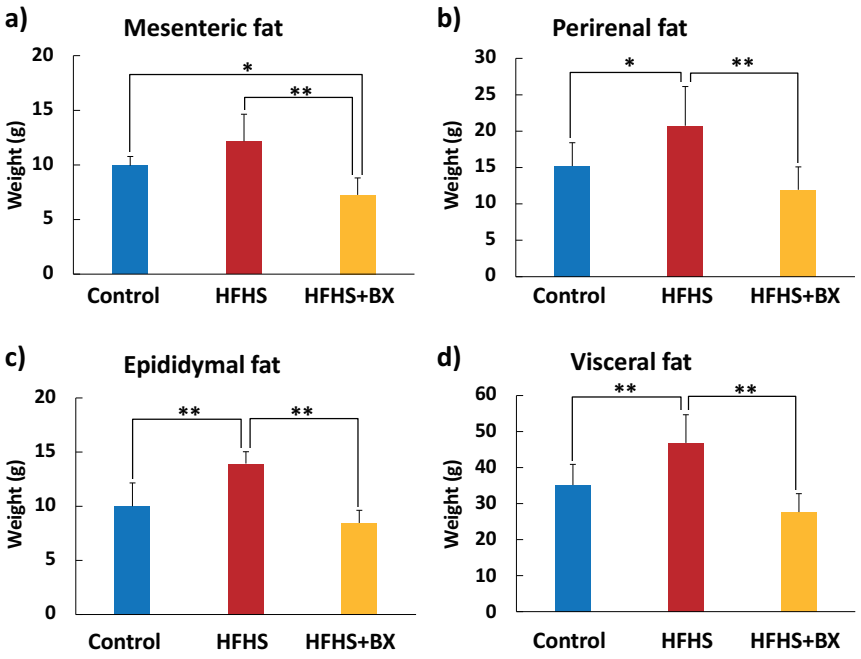

Fig. 5. **Visceral fat.**

Weight of **a)** mesenteric fat, **b)** perirenal fat, **c)** epididymal fat, and **d)** visceral fat. Visceral fat refers to total fat comprising mesenteric, perirenal and epididymal fat. Results are expressed as mean ± SD; *p < 0.05, **p < 0.01 by the Tukey-Kramer test. Control, n = 8; HFHS, high-fat high-sugar, n = 9; HFHS+BX, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 6; BX, *Basidiomycetes-X*; SD, standard deviation.

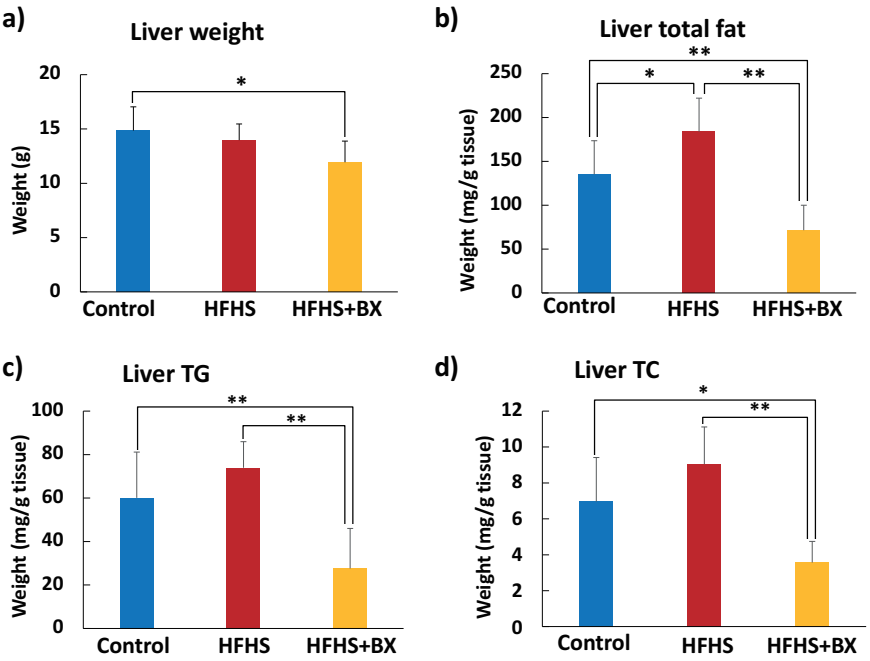

Fig. 6. **Fat in liver tissue.**

a) Liver weight, **b)** total fat, **c)** TG and **d)** TC levels. Total fat refers to the sum of TG and TC. Results are expressed as mean ± SD; *p < 0.05, **p < 0.01 by the Tukey-Kramer test. Control, n = 8; HFHS, high-fat high-sugar, n = 9; HFHS+BX, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 6; TG, triglyceride; TC, total cholesterol; BX, *Basidiomycetes-X*; SD, standard deviation.

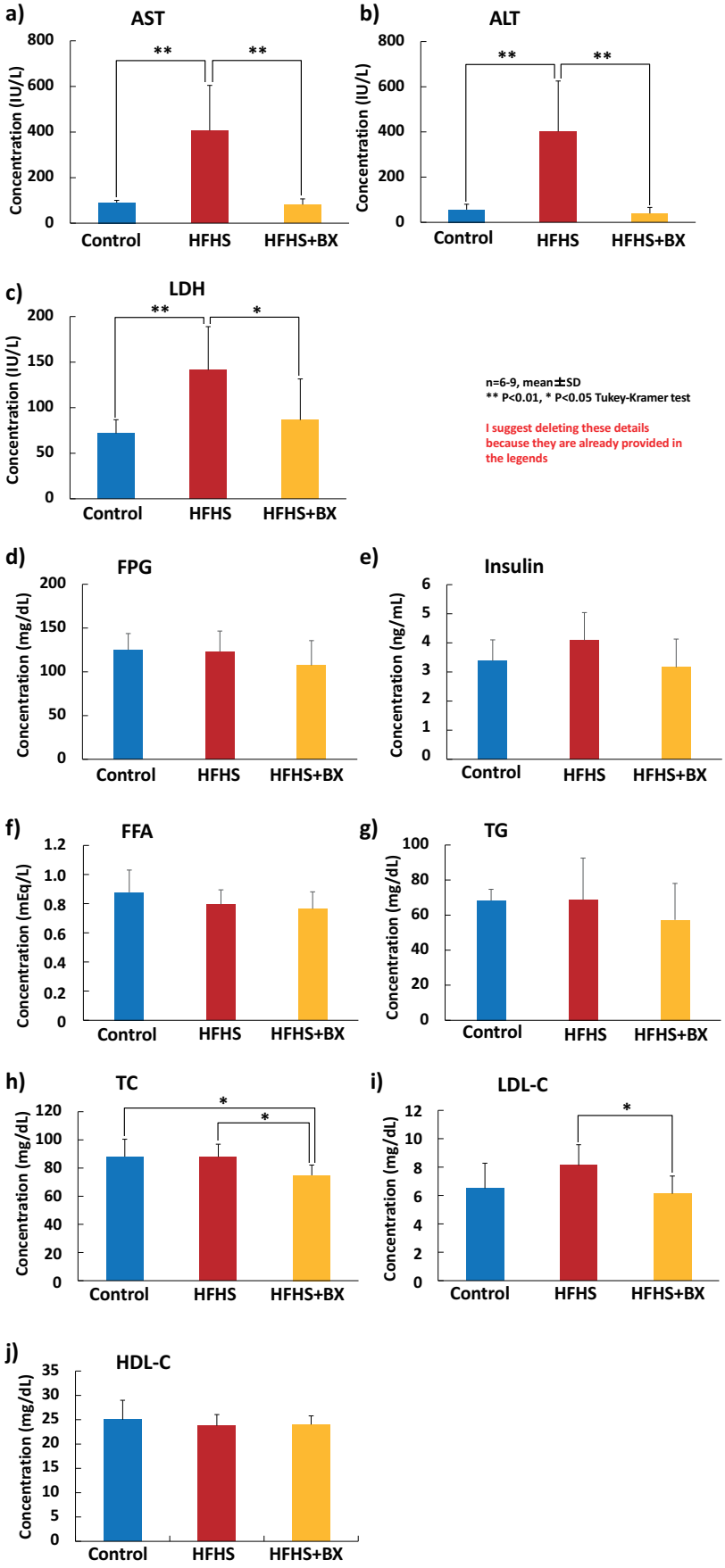

Fig. 7. **Blood chemistry.**

Levels of **a)** AST, **b)** ALT, **c)** LDH, **d)** FPG, **e)** insulin, **f)** FFA, **g)** TG, **h)** TC, **i)** LDL-C, and **j)** HDL-C. Results are expressed as mean ± SD; *p < 0.05, **p < 0.01 by the Tukey-Kramer test. Control, n = 8; HFHS, high-fat high-sugar, n = 9; HFHS+BX, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 6; AST, aspartate transaminase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; FPG, fasting plasma glucose; FFA, free fatty acid; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; BX, *Basidiomycetes-X*; SD, standard deviation.

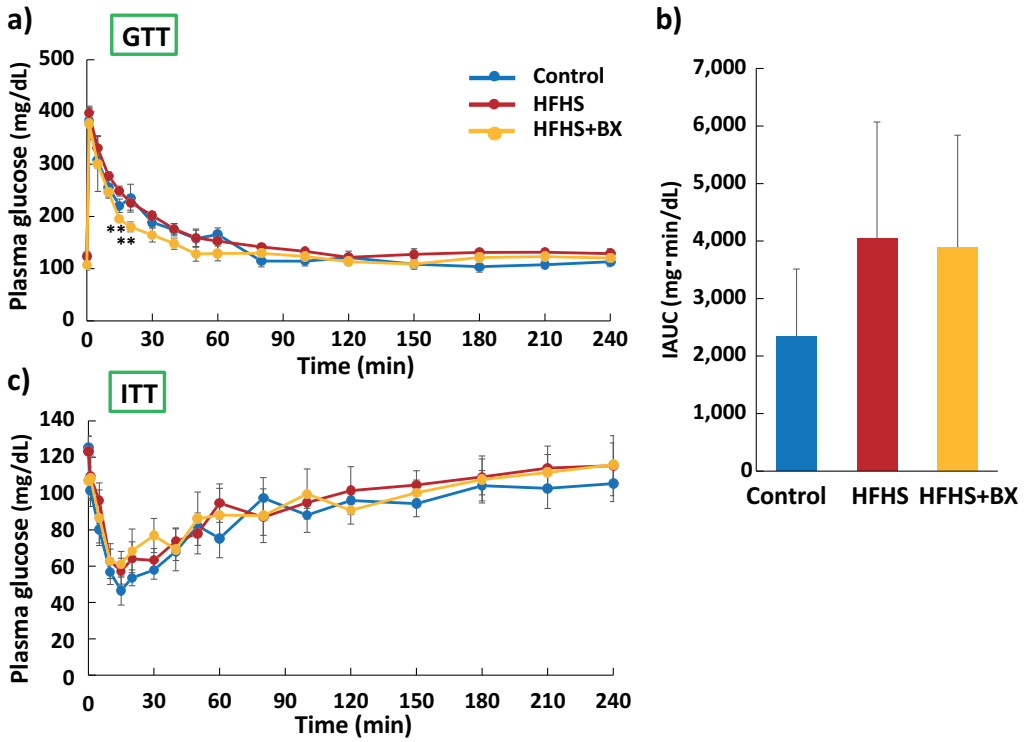

Fig. 8. **Glucose in GTT and ITT.**

a) Change in plasma glucose in GTT, **b)** the iAUC, and **c)** change in plasma glucose in ITT. Results are expressed as mean ± SEM; **p < 0.01 by Student's t test vs HFHS. Control, n = 4; HFHS, high-fat high-sugar, n = 4; HFHS+BX, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 4; GTT, glucose tolerance test; ITT, insulin tolerance test; iAUC, incremental area under the curve; BX, *Basidiomycetes-X*; SEM, standard error mean.

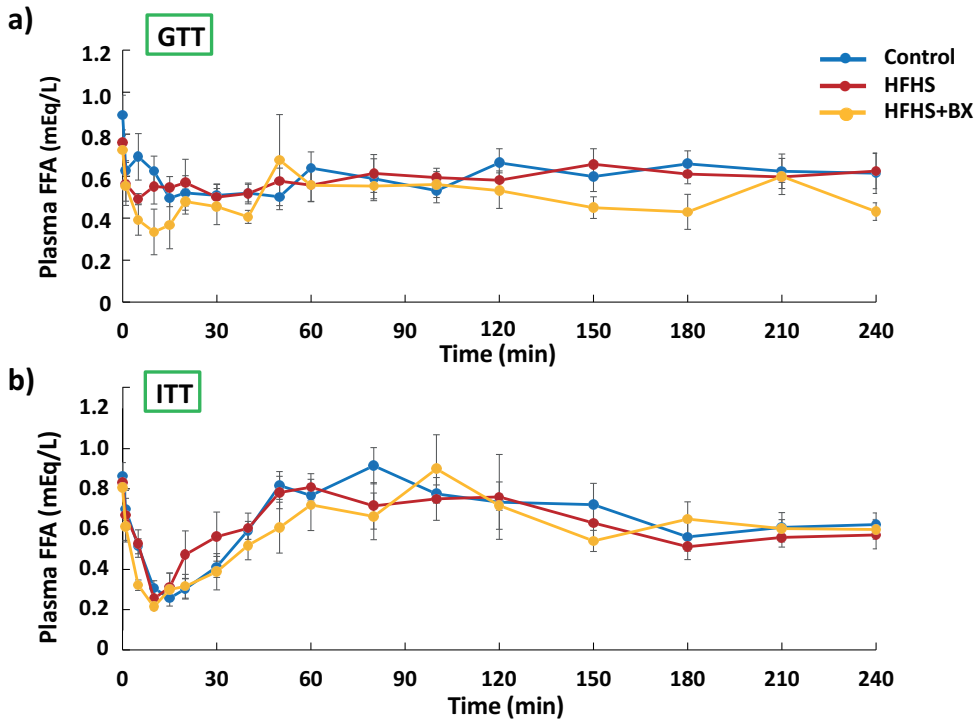

Fig. 9. **FFA in GTT and ITT.**

Changes in plasma FFA in **a)** GTT and **b)** ITT. Results are expressed as mean ± SEM. Control, n = 4; HFHS, high-fat high-sugar, n = 4; HFHS+BX, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 4; GTT, glucose tolerance test; ITT, insulin tolerance test; BX, *Basidiomycetes-X*; SEM, standard error mean.

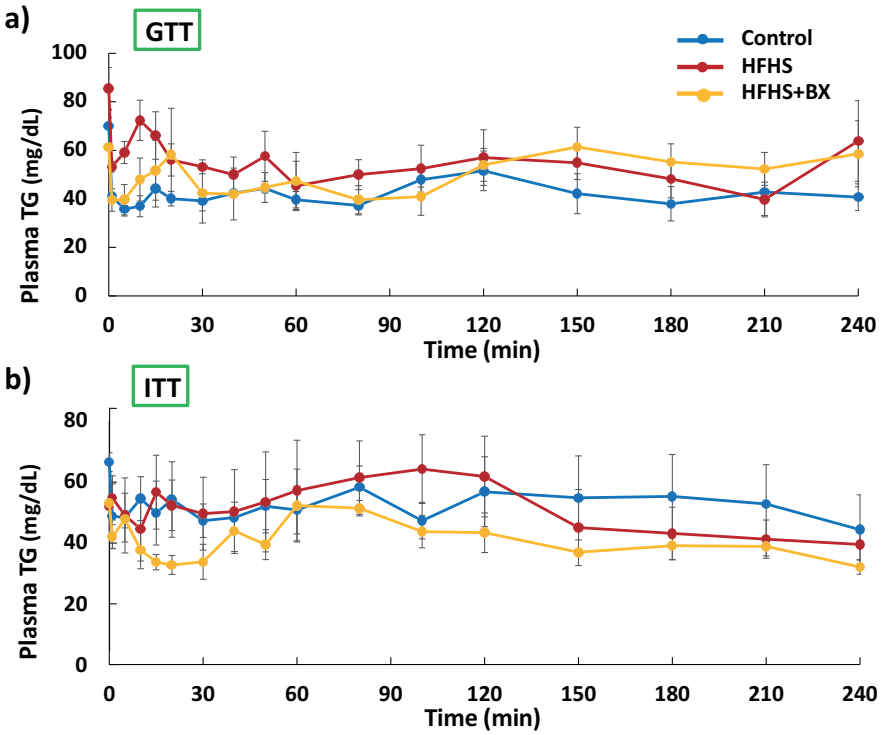

Fig. 10. **TG in GTT and ITT.**

Changes in plasma TG in **a)** GTT and **b)** ITT. Results are expressed as mean ± SEM. Control, n = 4; HFHS, high-fat high-sugar, n = 4; HFHS+BX, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 4; GTT, glucose tolerance test; ITT, insulin tolerance test; BX, *Basidiomycetes-X*; SEM, standard error mean.

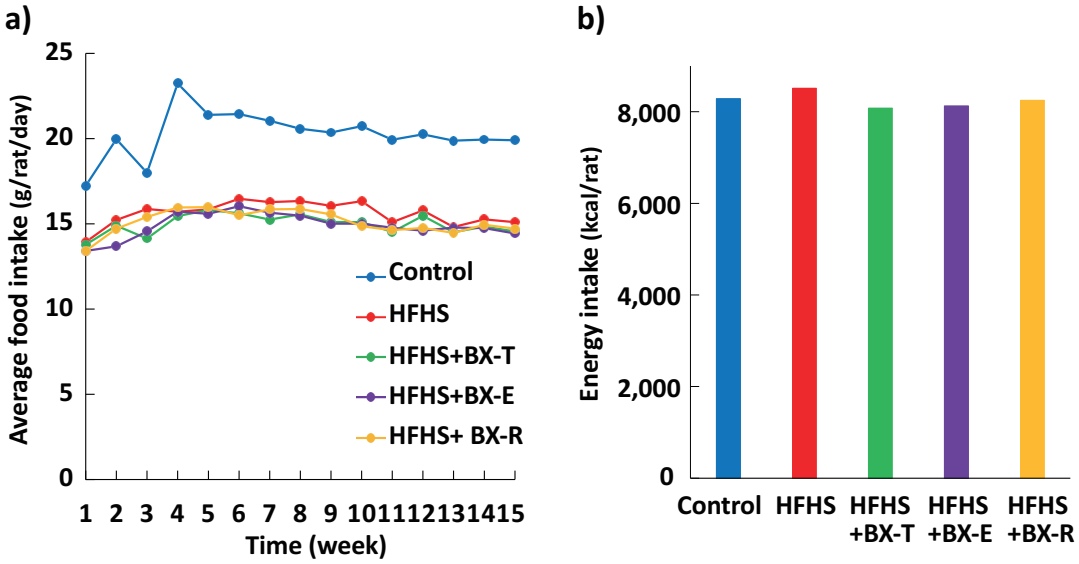

Fig. 11. **Food and energy intake.**

a) Food intake and **b)** energy intake during the experiment. Control, n = 10; HFHS, high-fat high-sugar, n = 10; HFHS+BX-T, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 10; HFHS+BX-E, high-fat high-sugar feed supplemented with 5% BX water extract, n = 10; HFHS+BX-R, high-fat high-sugar feed supplemented with 5% BX extract residue, n = 8.

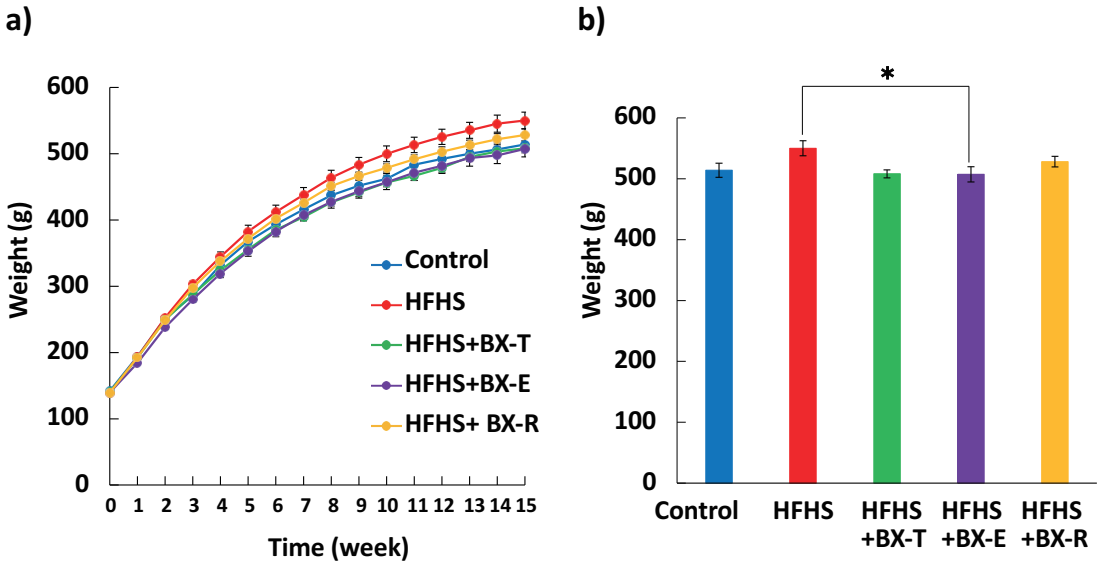

Fig. 12. **Body weight.**

a) Change in body weight and **b)** body weight at the end of the experiment. Control, n = 10; HFHS, high-fat high-sugar, n = 10; HFHS+BX-T, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 10; HFHS+BX-E, high-fat high-sugar feed supplemented with 5% BX water extract, n = 10; HFHS+BX-R, high-fat high-sugar feed supplemented with 5% BX extract residue, n = 8. Results are expressed as mean ± SEM; *p < 0.05 by the Tukey-Kramer test. SEM, standard error mean.

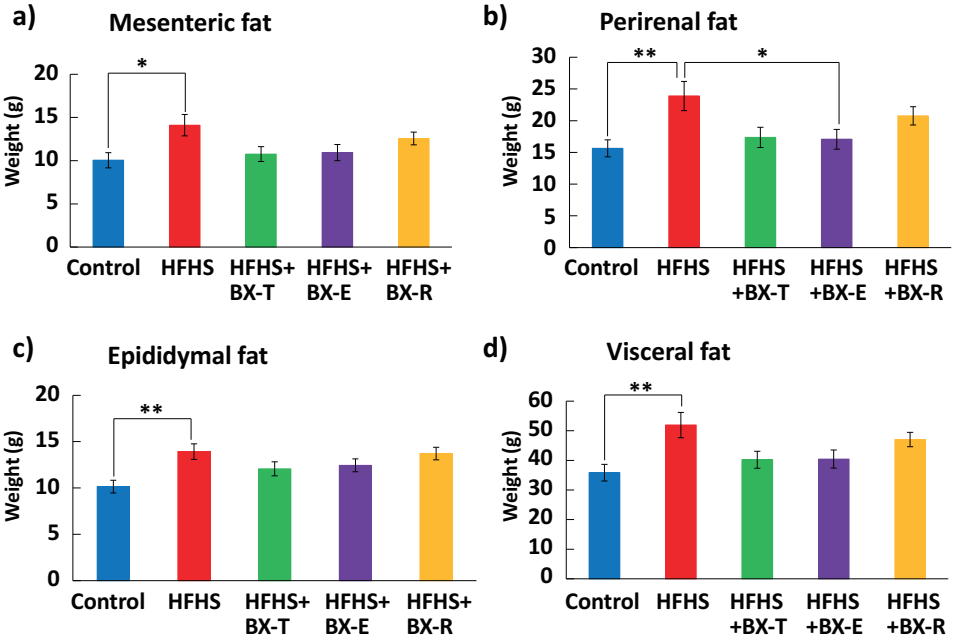

Fig. 13. **Visceral fat.**

Weight of **a)** mesenteric fat, **b)** perirenal fat, **c)** epididymal fat and **d)** visceral fat. Visceral fat refers to total fat comprising mesenteric, perirenal and epididymal fat. Control, n = 10; HFHS, high-fat high-sugar, n = 10; HFHS+BX-T, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 10; HFHS+BX-E, high-fat high-sugar feed supplemented with 5% BX water extract, n = 10; HFHS+BX-R, high-fat high-sugar feed supplemented with 5% BX extract residue, n = 8. Results are expressed as mean ± SEM; *p < 0.05, **p < 0.01 by the Tukey-Kramer test. SEM, standard error mean.

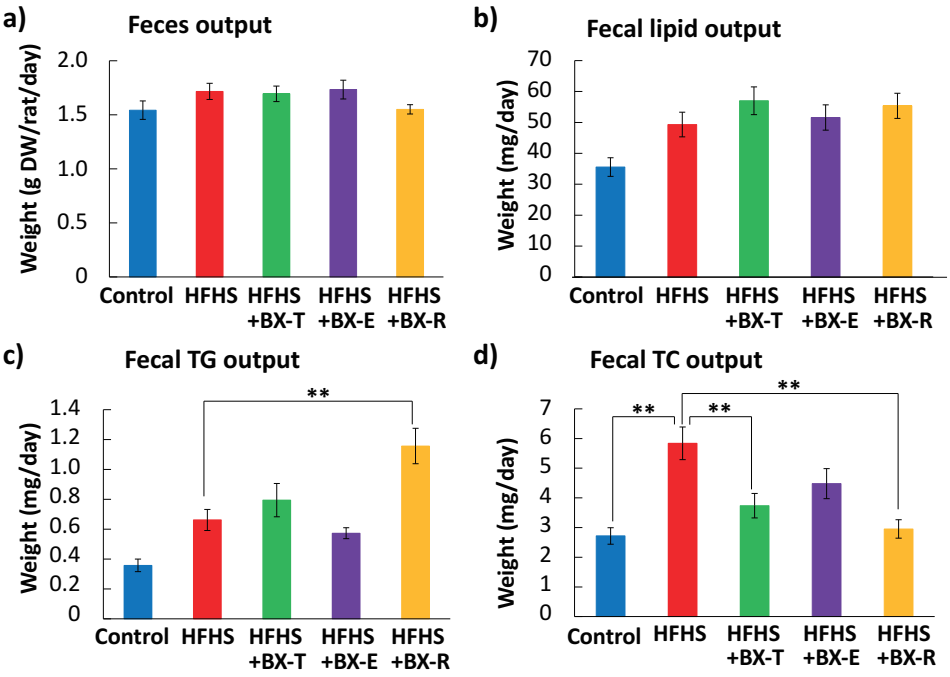

Fig.14. **Feces lipid output.**

a) Feces output, **b)** fecal lipid output, **c)** fecal TG output, and **d)** fecal TC output. Control, n = 10; HFHS, high-fat high-sugar, n = 10; HFHS+BX-T, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 10; HFHS+BX-E, high-fat high-sugar feed supplemented with 5% BX water extract, n = 10; HFHS+BX-R, high-fat high-sugar feed supplemented with 5% BX extract residue, n = 8. Results are expressed as mean ± SEM; **p < 0.01 by the Tukey-Kramer test. TG, triglyceride; TC, total cholesterol; SEM, standard error mean.

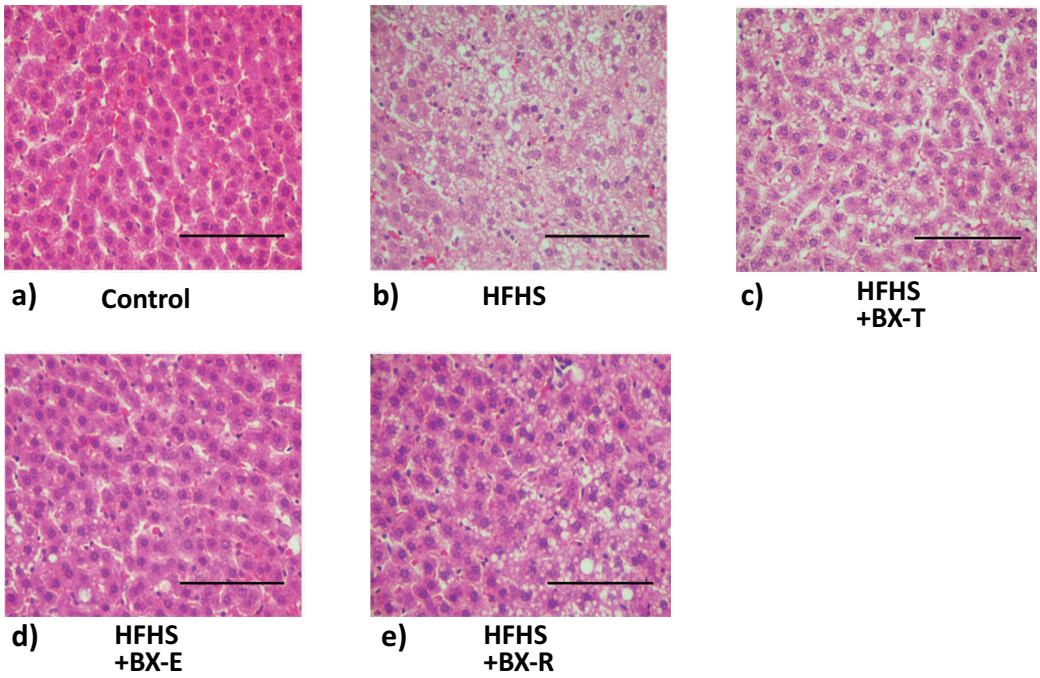

Fig.15. **Liver histology.**

a) Control, **b)** HFHS, **c)** HFHS+BX-T, **d)** HFHS+BX-E, and **e)** HFHS+BX-R. Scale bars indicate 100 μm. Hematoxylin and eosin stain. HFHS, high-fat high-sugar; HFHS+BX-T, high-fat high-sugar feed supplemented with 5% BX dry powder; HFHS+BX-E, high-fat high-sugar feed supplemented with 5% BX water extract; HFHS+BX-R, high-fat high-sugar feed supplemented with 5% BX extract residue.

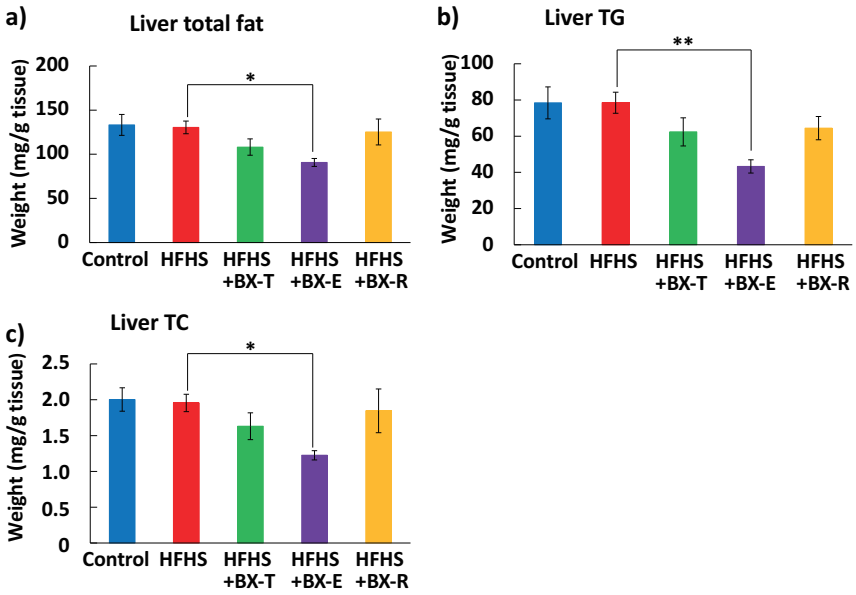

Fig. 16. **Fat in liver tissue.**

a) Total fat, **b)** TG, and **c)** TC levels. Total fat refers to the sum of TG and TC. Control, n = 10; HFHS, high-fat high-sugar, n = 10; HFHS+BX-T, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 10; HFHS+BX-E, high-fat high-sugar feed supplemented with 5% BX water extract, n = 10; HFHS+BX-R, high-fat high-sugar feed supplemented with 5% BX extract residue, n = 8. Results are expressed as mean ± SEM; *p < 0.05, **p < 0.01 by the Tukey-Kramer test vs HFHS. SEM, standard error mean.

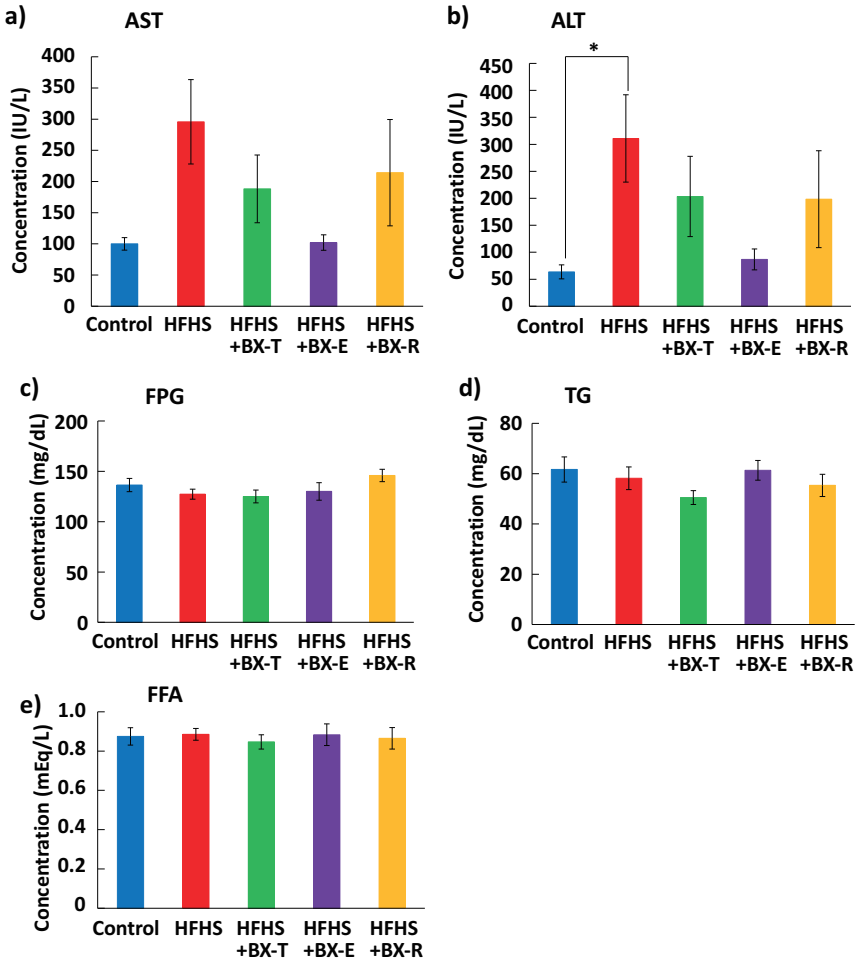

Fig. 17. **Blood chemistry.**

a) AST, **b)** ALT, **c)** FPG, **d)** TG, and **e)** FFA. Control, n = 10; HFHS, high-fat high-sugar, n = 10; HFHS+BX-T, high-fat high-sugar feed supplemented with 5% BX dry powder, n = 10; HFHS+BX-E, high-fat high-sugar feed supplemented with 5% BX water extract, n = 10; HFHS+BX-R, high-fat high-sugar feed supplemented with 5% BX extract residue, n = 8. Results are expressed as mean ± SEM; *p < 0.05, **p < 0.01 by the Tukey-Kramer test vs HFHS. AST, aspartate transaminase; ALT, alanine aminotransferase; FPG, fasting plasma glucose; TG, triglyceride; FFA, free fatty acid; SEM, standard error mean.

References

1) Mycology Techno Co., Ltd., Nomoto K, Watanabe T. Composition against atopic disease. Patent No. WO2008129996. 2008-10-30.
2) Watanabe T, Nakajima Y, Konishi T. In vitro and *in vivo* antioxidant activity of hot water extract of basidiomycetes-X, newly identified edible fungus. Biol Pharm Bull. 2008; 31: 111-117.
3) Kozuka C, Yabiku K, Sunagawa S, et al. Brown rice and its components, γ-oryzanol, attenuate the preference for high fat diet by decreasing hypothalamic endoplasmic reticulum stress in mice. Diabetes. 2012; 61:3084-3093.
4) Masuzaki H, Kozuka C, Yamamine M, et al. Brown rice-specific γ-oryzanol-based novel approach toward lifestyle-related dysfunction of brain and impaired glucose metabolism. Glycative Stress Res. 2017; 4: 58-66.
5) Kozuka C, Shimizu-Okabe C, Takayama C, et al. Marked augmentation of PLGA nanoparticle-induced metabolically beneficial impact of γ-oryzanol on fuel dyshomeostasis in genetically obese-diabetic ob/ob mice. Drug Deliv. 2017; 24: 558-568.
6) Nakamura A, Terauchi Y. Factors associated with the glucose-lowering effect of vildagliptin identified from the results of the oral glucose tolerance test in Japanese patients with type 2 diabetes. Endocr J. 2013; 60: 45-49.
7) Suzuki D, Toyoda M, Kimura M, et al. Effects of liraglutide, a human glucagon-like peptide-1 analogue, on body weight, body fat area and body fat-related markers in patients with type 2 diabetes mellitus. Intern Med. 2013; 52: 1029-1034.
8) Yabe D, Seino Y, Fukushima M, et al. β cell dysfunction versus insulin resistance in the pathogenesis of type 2 diabetes in East Asians. Curr Diab Rep. 2015; 15: 602.
9) Kozuka C, Sunagawa S, Ueda R, et al. γ-Oryzanol protects pancreatic β-cells against endoplasmic reticulum stress in male mice. Endocrinology. 2015; 156:1242-1250.
10) Kozuka C, Sunagawa S, Ueda R, et al. A novel insulinotropic mechanism of whole grain-derived γ-oryzanol via the suppression of local dopamine D2 receptor signaling in mouse islet. Br J Pharmacol. 2015; 172: 4519-4534.
11) Tolhurst G, Heffron H, Lam YS, et al. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes. 2012; 61: 364-371.
12) Tang C, Ahmed K, Gille A, et al. Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. Nat Med. 2015; 21: 173-177.
13) McNelis JC, Lee YS, Mayoral R, et al. GPR43 potentiates β-cell function in obesity. Diabetes. 2015; 64: 3203-3217.
14) DiBaise JK, Zhang H, Crowell MD, et al. Gut microbiota and its possible relationship with obesity. Mayo Clin Proc. 2008; 83: 460-469.
15) Sefcíková Z, Kmet V, Bujnáková D, et al. Development of gut microflora in obese and lean rats. Folia Microbiol (Praha). 2010; 55: 373-375.
16) Kim KA, Gu W, Lee IA, et al. High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway. PLoS One. 2012; 7: e47713.
17) Yonei Y, Yagi M, Nakamura M, et al. Effects of astaxanthin on intestinal microflora in mice fed a high-fat diet. Anti-Aging Med. 2013; 10: 77-91.
18) Sato J, Kanazawa A, Ikeda F, et al. Gut dysbiosis and detection of "live gut bacteria" in blood of Japanese patients with type 2 diabetes. Diabetes Care. 2014; 37: 2343-2350.
19) Oishi Y, Manabe I. Adipose stem cell system. Clin Calcium. 2017; 27: 795-801. (in Japanese)
20) Minokoshi Y. Central regulation of appetite and energy metabolism. Clin Calcium. 2018; 28: 45-55. (in Japanese)
21) Sakai T. Epigenetic research in obesity and life-style-related diseases. Journal of Clinical and Experimental Medicine (Igaku no Ayumi). 2019; 268: 359-364. (in Japanese)
22) Ikeda K, Kajimura S. Pathogenesis of obesity: Obesity and brown / beige adipose tissue. The Medical Frontline. 2019; 74: 60-66. (in Japanese)
23) Ono H. Why is the insulin resistance induced? : Distribution and function of brown adipose tissue influen23) cing insulin sensitivity. Experimental Medicine. 2017; 35: 217-224. (in Japanese)