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Dietary Bamboo Charcoal Decreased Visceral Adipose Tissue Weight by Enhancing Fecal Lipid Excretions in Mice with High-Fat Diet-Induced Obesity

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ABSTRACT: Bamboo charcoal (BC) powder is prepared from thick bamboo stems via dry distillation and is often used for food coloring. Due to the unique structure of the micropores in bamboo stems, BC powder also serves as an indigestible carrier to prevent the absorption of toxic substances and nutrients from the digestive tract. This study evaluated the health-promoting function of BC, particularly its effects in decreasing visceral adipose tissue in a mouse model with high-fat diet (HFD)-induced obesity. Four-week-old male C57BL/6J mice were divided into three groups and fed either a low-fat (LF) diet (7% fat), HF diet (25% fat), or HF diet with 0.5% BC (HF-BC). After 80 days, the HF-BC diet was found to have decreased epididymal and mesenteric white adipose tissue weights compared to HFD. The inhibition of visceral fat accumulation by BC intake was partly due to enhanced fecal fatty acid excretion induced by its bile acid-binding and pancreatic lipase inhibition. Contrarily, the gut microbiota, known to influence systemic energy metabolism, did not change significantly between the HF and HF-BC groups. These results indicate that dietary BC inhibits visceral fat accumulation, which could reduce obesity development.

Keywords: bamboo charcoal, intestinal absorption, obesity, visceral adipose tissue

INTRODUCTION

Bamboo is familiar to Asian life and is recognized as a symbol of Asian sentiment. As bamboo is widely distributed in Asia, there is much room for its utilization and development. As bamboo charcoal (BC) can be manufactured by dry distillation of bamboo at high temperatures, it is plentiful, cheap, easy to prepare, and relatively non-toxic (Teraoka et al., 2004). Unlike charcoal that is primarily used as fuel, BC can remove formaldehyde (Suresh and Bandosz, 2018), regulate humidity (Horikawa et al., 2010), and purify water (Lin et al., 2017).

Food coloring agents can stimulate the appetite as a food additive (Cho et al., 2015; Foroni et al., 2016). For example, squid ink has been used as black coloring in food in Japan. However, because squid has been categorized as allergenic and squid populations are decreasing, alternatives have been considered (Taylor, 2008). BC is a food additive that has been approved for use as a black food coloring and is designated as an existing additive in Japan. BC powder, as a replacement for squid ink, is increasingly used as food coloring in bread, pasta, and other products.

Although many studies on the performance of BC for treating poisoning have been documented (Van et al., 2006), few reports regarding its health-promoting functions are available. As BC powder comprises black charcoal, a carbon allotrope with almost no digestible and absorbable components, it is used as a coloring agent for evaluating intestinal motility (Fujisaka et al., 2020). Therefore, BC might possess the same health-promoting functions as insoluble dietary fiber. For example, insoluble dietary fiber promotes laxation in humans (Dahl et al., 2005) and suppresses the rise of postprandial blood glucose levels (Takano et al., 2013). Insoluble dietary fiber also possesses serum cholesterol-lowering effects in mice and humans (Ausar et al., 2003; van Bennekum et al., 2005). The paper also studies the composition of the gut microbiota that is significantly affected by dietary fiber (Benus et al., 2010), and if changes in the gut micro-

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biota affect energy harvesting and body weight (BW) (Turnbaugh et al., 2006).

In this study, we evaluated the potential health-promoting functions of BC, particularly its effects on decreasing visceral adipose tissue weight, using a mouse model of high-fat diet (HFD)-induced obesity.

MATERIALS AND METHODS

Materials

BC powder was obtained from Kandagiko Co., Ltd.. Moso bamboo from Japanese prefecture Tottori was used as the raw material. The bamboo was cut into 4 m lengths and put into a pot, then the bamboo was directly ignited using a gas burner. After confirming that the bamboo was sufficiently heated, the pot was covered and carbonized at approximately 1,000°C for 5 days. After carbonization, pulverized coal pieces >6 mm were removed using a vibrating sieve, and BC pieces of 1∼6 cm were recovered. The BC was coarsely pulverized to a particle size of 4 mm or less, sterilized, and finely pulverized to a particle size of ≤45 μm. Water, crude protein, crude ash, and crude fat contents of BC powder were determined according to the Official Methods of Analysis of AOAC International (2019). The particle sizes of BC were determined using a laser micron sizer (LMS-2000e, SEISHIN ENTERPRISE Co., Ltd.), and the specific surface area, total pore volume, and the average pore size were measured using gas-sorption surface area and pore size analyzer (QUADRASORB evo 4, Anton Paar). The BC powder contained 8.9 wt% moisture, 1.5 wt% crude protein, 6.8 wt% crude ash, and no crude fat. The average particle size was 40.6 μm, the specific surface area was 175 m^2/g, the total pore volume was 0.110 mL/g, and the average pore size was 2.52 nm.

Soybean oil (Merck KGaA), lard (Junsei Chemical Co., Ltd.), L-cystine (Tokyo Chemical Industry Co., Ltd.), choline bitartrate (Nacalai Tesque, Inc.), *tert*-Butylhydroquinone (Fujifilm Wako Pure Chemical Corp.), and other substances (Oriental Yeast Co., Ltd.) were used as ingredients for the experimental diets. Other chemicals used were special grade products.

Animals and experimental approval

The 24 four-week-old male C57/BL6J mice (Japan SLC, Inc.) were kept in a breeding rack (KN-735-XX, Natsume Seisakusho Co., Ltd.) at 20∼22°C (lit from 8 AM to 8 PM). The experimental diets and drinking water were provided *ad libitum*. This study was reviewed and approved by the Animal Ethics Committee of Kansai University with the "Guide for the Care and Use of Experimental Animals issued by the Prime Minister's Office of Japan" (approval no. 2112).

Animal diets and care

The mice were divided into three groups [low-fat (LF), HF, and HF-BC groups], each containing eight mice with a similar average BW after a 7-day acclimatization period. Mice in the LF and HF groups were given the American Institute of Nutrition (AIN)-93G formula diet (Reeves et al., 1993) and AIN-93G formula-modified HF diet (22 wt% lard and 3 wt% soybean oil), respectively. Mice in the HF-BC group were fed an HF diet with cellulose replaced by BC at 0.5 wt%. The ingredients of each of the experimental diets are shown in Table 1.

One day prior to euthanizing, feces from each mouse were collected, weighed, lyophilized, and ground using a conventional grinder. After feeding the mice with the experimental diet for 80 days, the mice that had not fasted were anesthetized with isoflurane and euthanized with blood collecting (9 AM to 12 PM). Blood was centrifuged at 2,000 *g* for 15 min to obtain serum. The organs (liver, kidney, spleen, cecum) along with tissues [epididymal white adipose tissue (WAT), perirenal WAT, mesenteric WAT, and inguinal WAT] were removed from the body and rinsed with cold saline. The organs were weighed on an electronic balance, frozen in liquid nitrogen, and stored in a deep freezer (−80°C).

Biochemical analysis of serum

Serum biochemical parameters, including alanine aminotransferase, albumin, albumin/globulin, aspartate aminotransferase, creatinine kinase, high-density lipoprotein cholesterol (HDL-C), lactate dehydrogenase, non HDL-C, phospholipid (PL), serum urea nitrogen, total cholesterol, total lipid, total protein, and triglyceride (TG) were measured using an Olympus AU5431 automatic analyzer (Olympus Corp.) by Japan Medical Laboratory.

Table 1. Ingredients in the experimental diets

Ingredients (g/kg)	LF	HF	HF-BC
Dextrinized corn starch	132	87	87
Corn starch	397.486	262.486	262.486
Sucrose	100	100	100
Cellulose	50	50	45
BC			5
Casein	200	200	200
L-Cystine	3	3	3
Choline bitartrate	2.5	2.5	2.5
AIN-93G mineral mixture	35	35	35
AIN-93 vitamin mixture	10	10	10
Soybean oil	30	30	30
Lard	40	220	220
tert-Butylhydroquinone	0.014	0.014	0.014

LF, low-fat; HF, high-fat; BC, bamboo charcoal; AIN, American Institute of Nutrition.

Lipid analysis of the liver and feces

Total lipids in the liver were extracted using the Bligh and Dyer method (Bligh and Dyer, 1959). The total lipid solution dissolved in 2-propanol was used to measure TG content using the Wako Triglyceride E-Test (Fujifilm Wako Pure Chemical Corp.) according to the manufacturer's instructions. After saponification with sodium hydroxide, the cholesterol content in the liver was analyzed using a gas chromatography equipped with an SH-Rtx-5MS column (Product No. 12623, Shimadzu GLC Ltd.). 5α-Cholestane was used as an internal standard (Kaneda et al., 1980). Finally, liver PL content was measured using a phosphorus assay after wet ashing (Rouser et al., 1970).

The fecal neutral sterol (cholesterol and coprostanol) and total bile acid (BA) contents were measured prior to gas chromatography and total BAs testing (Fujifilm Wako Pure Chemical Corp.), respectively. Fecal total sterol content was calculated as the sum of neutral sterol and total BA. Fecal total fatty acid (FA) content was measured by methods described by Van de Kamer et al. (1949).

16S rRNA amplicon sequencing

Five fecal samples were randomly selected from each of the LF, HF, and HF-BC groups. According to the manufacturer's protocol, genomic DNA from the fecal samples was extracted using ISOSPIN Fecal DNA (Nippon Gene Co., Ltd.).

The V3 and V4 hypervariable regions of the 16S rRNA were amplified using the primer set 341F/806R (Hjelmsø et al., 2014). All polymerase chain reaction products were purified using Agencourt AMPure XP (Beckman Coulter, Inc.) and sent to GenomeLead Co., Ltd. for metagenomic sequencing using Illumina next-generation sequencing platform (Illumina, Inc.).

Bioinformatic analysis

Bioinformatic analysis using QIIME2 (version 2022.8) included the quality control of the paired-end 300-bp reads and featured table construction using the DADA2 plugin. The classification table was obtained by matching against the Greengenes database and applying the QIIME2 feature classifier plugin. Principal coordinate analysis (PCoA) based on Bray-Curtis distance was visualized using EMPeror (Vázquez-Baeza et al., 2013).

BA-binding capacity analysis

The determination of BA-binding capacity was based on the methods described by Higaki et al. (2006), with some modifications. In brief, 2 mL of 0.1 M phosphate buffer (pH 7.4) containing 200 μM of cholic acid (CA) or taurocholic acid (TCA) was added to 50 mg of BC powder or cellulose and shaken at 37°C for 1 h. Then, the BA content in the supernatant obtained by centrifugation at 16,000 *g* for 5 min was analyzed using a BA assay kit (total bile acids test, Wako). The difference in CA and TCA contents before and after adding BC or cellulose was used to determine the amount of CA and TCA bound per sample weight as BA-binding capacity.

Pancreatic lipase inhibition activity

Pancreatic lipase inhibition activity in the presence of BC and cellulose was determined as described previously (Tsujita et al., 2003), with some modifications. In brief, 0.5 mL of the pancreatic lipase reaction solution [100 unit/mL pancreatic lipase, 10 μM trioleoylglycerol, 1.4 μM soybean phosphatidylcholine, and 1.05 μM of TCA in 100 mM N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid and NaCl (pH 7.0)] was added to 10 mg of BC or cellulose and the mixture was incubated at 37°C for 30 min. The amount of free FA was determined using a NEFA C-test (Fujifilm Wako Pure Chemical Corp.). Pancreatic lipase inhibitory activity (%) was calculated using the following equation:

pancreatic lipase inhibitory activity (%)=100−{(A0−Ac/A0)×100}, where Ac refers to the absorbance value of the reaction solution with porcine lipase and BC or cellulose, and A0 refers to the absorbance of the reaction solution without BC or cellulose.

Statistical analysis

Data were expressed as mean±standard error. In the *in vivo* study, differences between the LF and HF groups and between the HF and HF-BC groups were evaluated using Dunnett's multiple comparison test. In the *in vitro* study, the differences were evaluated using Student's *t*-test. The relationship between fecal FA and BA excretions was evaluated using Peason's correlation coefficient test. The statistical significance and statistical tendency were set at $P<0.05$ and $0.05\leq P<0.10$, respectively. All statistical tests were performed using GraphPad Prism version 7.0

Table 2. Growth parameters and relative organ weights

	Experimental groups		
	LF	HF	HF-BC
Growth parameters			
Initial BW (g)	18.5±0.3	18.5±0.3	18.5±0.5
Final BW (g)	28.2±0.7*	33.7±1.0	32.6±1.3
BW gain (g/d)	0.12±0.01*	0.19±0.01	0.19±0.01
Food intake (g/d)	2.8±0.0	2.8±0.1	2.8±0.1
Relative organ weight (g/100 g BW)			
Liver	4.04±0.14*	3.34±0.14	3.72±0.04*
Kidney	1.13±0.04	1.01±0.01	1.22±0.15
Spleen	0.21±0.02	0.21±0.02	0.22±0.02
Cecum	1.30±0.10*	0.92±0.05	0.94±0.07

Values are presented as mean±SE (n=8).
*$P<0.05$ vs. HF group (Dunnett's multiple comparison test).
LF, low-fat; HF, high-fat; BC, bamboo charcoal; BW, body weight.

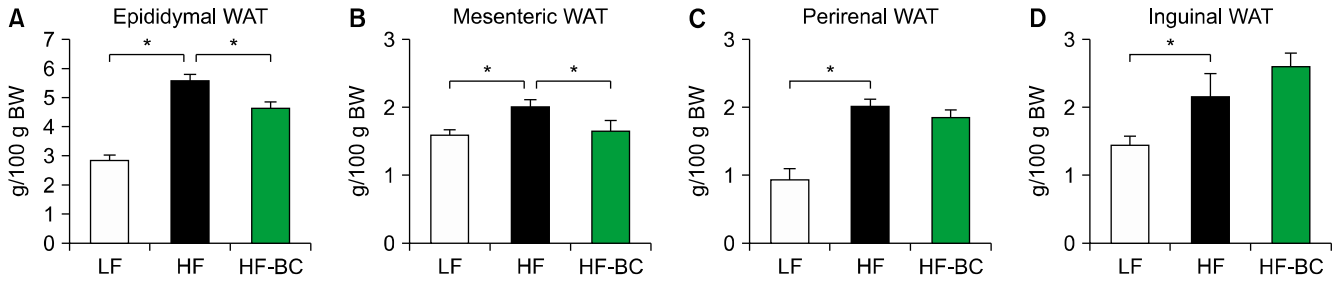

Fig. 1. Relative white adipose tissue (WAT) weight. (A) Relative epididymal WAT weight. (B) Relative mesenteric WAT weight. (C) Relative perirenal WAT weight. (D) Relative inguinal WAT weight. Data are presented as mean±SE (n=8). **P*<0.05 vs. high-fat (HF) group (Dunnett's multiple comparison test). BW, body weight; LF, low-fat; BC, bamboo charcoal.

software (GraphPad Software).

RESULTS AND DISCUSSION

The growth parameters and relative organ weights of the mice are presented in Table 2, and the relative WAT weights are shown in Fig. 1. Although the initial BW and food intake were not significantly different among the groups, the final BW and BW gains in the HF group were significantly higher than those in the LF group. Moreover, the HF diet group had significantly decreased relative liver and cecum weights and increased relative weight of WAT from epididymal, mesenteric, perirenal, and inguinal tissues compared with mice who were fed the LF diet. Therefore, the decreased relative liver and cecum weights in the HF group could be related to the increased BW. Moreover, WAT weights were increased in the HF group compared to the LF group, contributing to the final BW (Fig. 1).

C57BL/6J mice, an obesity-prone strain, can develop diet-induced obesity by feeding an HF diet (Nishikawa et al., 2007). Under our experimental conditions, the HF group showed an increased final BW and relative WAT weight, confirming that the HF diet could induce obesity in our experimental mice. In contrast, the HF-BC diet significantly decreased the relative epididymal and mesenteric WAT weights compared with the HF diet (Fig. 1). Epididymal, mesenteric, and perirenal WAT corresponded to visceral fat, and inguinal WAT corresponded to subcutaneous fat. Regarding subcutaneous fat and visceral fat obesity, the latter is closely related to metabolic syndromes such as diabetes, hypertension, and dyslipidemia (Matsuzawa et al., 2011). BC intake inhibits visceral fat accumulation, preventing the development of metabolic syndrome.

The lipid contents of the serum and liver of the mice are listed in Table 3. The serum total cholesterol, HDL-C, and non-HDL-C contents were significantly higher in the HF group compared to in the LF group. Moreover, the HF diet significantly increased liver TG and cholesterol contents compared with the LF diet.

Previous studies have shown that the lipid contents, including serum total cholesterol, HDL-C, and non-HDL-C contents, and liver TG and cholesterol contents increased in mice that developed obesity due to an HF diet (Sheng et al., 2019; Li et al., 2020; Kato et al., 2022). Mice in the HF group showed increased body and WAT weights and elevated serum and liver lipids. Liver TG content in the HF-BC group was lower than that in the HF group (*P*=

Table 3. Lipid content in the serum and liver

	Experimental groups		
	LF	HF	HF-BC
Serum (mg/dL)			
TG	82±19	55±5	60±9
PL	280±12	297±13	317±9
Total cholesterol	128±4*	154±9	160±6
HDL-C	100±2*	115±6	120±3
Non-HDL-C	28±2*	38±3	40±3
Liver (mg/g)			
TG	28.7±2.7*	54.4±8.7	39.4±5.0
PL	22.4±1.0	23.9±0.6	24.2±0.6
Cholesterol	2.1±0.1*	2.5±0.1	2.3±0.0

Values are presented as mean±SE (n=8).
**P*<0.05 vs. HF group (Dunnett's multiple comparison test).
LF, low-fat; HF, high-fat; BC, bamboo charcoal; TG, triglyceride; PL, phospholipid; HDL-C, high-density lipoprotein cholesterol.

Table 4. Serum biochemical parameters

	Experimental groups		
	LF	HF	HF-BC
Total protein (g/dL)	5.0±0.0	4.9±0.1	5.0±0.1
Albumin (g/dL)	3.0±0.0	2.9±0.0	3.0±0.0
A/G	1.5±0.0	1.4±0.0	1.5±0.0
AST (U/L)	42.8±0.6	47.9±2.0	48.1±4.9
ALT (U/L)	12.5±0.7	16.4±1.5	20.4±3.8
CPK (U/L)	25.4±1.1	29.6±2.3	32.9±3.4
LDH (U/L)	163±12	162±12	148±14
SUN (mg/dL)	30.0±2.0*	23.1±0.9	27.8±1.3

Values are presented as mean±SE (n=8).
**P*<0.05 vs. HF group (Dunnett's multiple comparison test).
LF, low-fat; HF, high-fat; BC, bamboo charcoal; A/G, albumin/globulin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CPK, creatinine kinase; LDH, lactate dehydrogenase; SUN, serum urea nitrogen.

0.090). Besides liver TG, serum and liver lipid and other serum biochemical parameters did not differ significantly between the HF and HF-BC groups (Table 4).

There are several possible mechanisms for the effect of dietary BC in reducing visceral fat accumulation. First, we evaluated the changes in the gut microbiota via 16S rRNA amplicon sequencing because the composition of the gut microbiota affects BW (Ley et al., 2005; Aoun et al., 2020). No significant differences were observed in the number of reads and α-diversity indices (Chao-1 and Simpson) among the groups. PCoA plots based on the Bray-Curtis distance and gut microbiota composition (Fig. 2A) showed that the HF and HF-BC groups formed a cluster and were distinctly separated from the cluster of the LF group (Fig. 2B).

Furthermore, changes in the gut microbiota composition at the phylum and genus level were associated with obesity. For example, the balance between the two phyla (Firmicutes and Bacteroidetes) is important in the regulation of BW (Ley et al., 2006), and the genus *Akkermansia* is associated with obesity (Hasani et al., 2021). However, this study showed no such differences (Fig. 2C and 2D). Furthermore, unlike soluble fiber, insoluble fiber has less effect on the colonic microbiota (Chen et al., 2019).

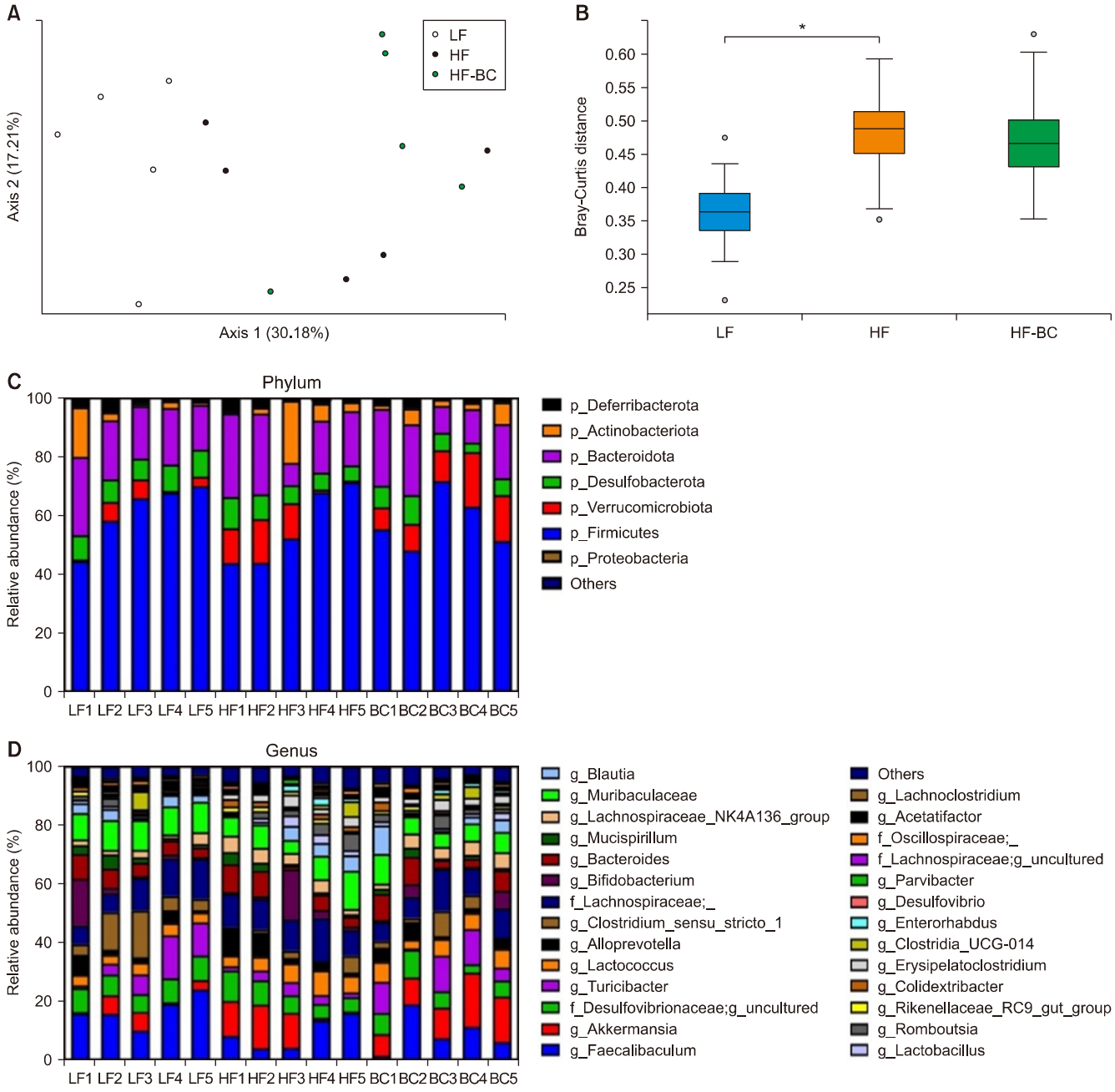

Fig. 2. Microbiota composition in the cecal content. (A) Principal coordinate analysis plot based on the Bray-Curtis distance. (B) Boxplot of Bray-Curtis distance. *P<0.05 (pairwise PERMANOVA). (C) Relative abundance of bacteria at the phylum level. (D) Relative abundance of bacteria at the genus level. LF, low-fat; HF, high-fat; BC, bamboo charcoal.

Table 5. Fecal sterol and fatty acid excretion

	Experimental groups		
	LF	HF	HF-BC
Neutral sterol (mg/d)[1)]	0.3±0.1*	1.2±0.2	1.0±0.2
Cholesterol	0.3±0.1*	1.1±0.2	1.1±0.1
Coprostanol	0.0±0.0	0.1±0.0	0.1±0.0
Total BA (mg/d)	0.3±0.0*	0.7±0.1	1.0±0.1*
Total sterol (mg/d)[2)]	0.6±0.1*	1.9±0.1	2.0±0.2
Total FA (mg/d)	6.0±0.5*	44.6±2.9	62.6±4.1*

Values are presented as mean±SE (n=8).
*P<0.05 vs. HF group (Dunnett's multiple comparison test).
[1)]Sum of cholesterol and coprostanol.
[2)]Sum of neutral sterol and total BA.
LF, low-fat; HF, high-fat; BC, bamboo charcoal; BA, bile acid; FA, fatty acid.

Therefore, nondigestible dietary BC inside the cecum may not affect the gut microbiota composition.

Enhanced fecal fat excretion is another possibility for suppressing the visceral fat accumulation of dietary BC (Stenkula et al., 2017). Therefore, we investigated the fecal neutral sterol, total BA, total sterol, and FA contents (Table 5) of the mice. We found that the contents in HF group were significantly higher than those in LF group. Some studies have shown that HF diet increase fecal lipids (neutral sterol, BA, and FA) excretion in mice (Oishi et al., 2015; Murakami et al., 2016; Wang et al., 2020). Therefore, increased fecal lipid excretion may be an effect of HF diet. Additionally, the HF-BC diet significantly increased the fecal total BA and FA excretions compared with the HF diet. Because conjugated BA is more efficiently reabsorbed in the ileum, activity of intestinal bile salt hydrolase (BSH) is involved in the removal of *N*-acyl amidation (glycine or taurine) of BA and is associated with fecal BA excretion (Tahri et al., 1997; de Aguiar Vallim et al., 2013). The BSH activity has been recorded in *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Enterococcus*, and *Lactobacillus* (Ridlon et al., 2016). However, this study showed no difference in the relative abundance of these microorganisms between the HF and HF-BC groups. Therefore, intestinal BSH activity is unlikely responsible for the increased fecal BA excretion in the HF-BC group. Contrarily, hydrophobic BC may adsorb BA by hydrophobic interaction in the gastrointestinal tract, enhancing its excretion. Therefore, we compared the BA-binding capacity of BC (Fig. 3) and identified significantly higher binding capacity for CA (unconjugated BA) and TCA (conjugated BA) compared to cellulose. BA contains hydrophobic and hydrophilic moieties, making them facially amphipathic (Mukhopadhyay and Maitra, 2004). The interaction between BA and insoluble fiber is dependent on the hydrophobicity of the BA, with viscosity having little interaction (Naumann et al., 2020). This indicates that the hydrophobic interaction between insoluble fiber and BA is related to the fiber structure. BC is hydrophobic and has a porous structure derived from vascular bundles, which vary in size from macropores (>50 nm), mesopores (2~50 nm), and micropores (<2 nm) (Isa et al., 2016). The hydrophobic ability and porous structure of BC may be involved in the BA-binding capacity, but a more detailed analysis using molecular docking is needed. Cellulose has been repeatedly shown to have low BA-binding capacity *in vitro* (Kahlon and Chow, 2000; Dongowski, 2007), and our results support this finding.

Lipase inhibitors such as orlistat, which increase fecal fat and induce body fat loss, are used to treat obesity (Sjöström et al., 1998). Fecal fat excretion could be an important mechanism for inducing body fat loss (Sjöström et al., 1998). In this study, we observed BC intake increased fecal FA excretion (FA content indicated as a measure of fat) and decreased relative epididymal and mesenteric WAT weights (Fig. 1). Therefore, decrease in relative visceral WAT weight due to BC intake may involve an enhancement of fecal FA excretion. Dietary fat cannot be absorbed by small intestinal epithelial cells without the action of pancreatic lipases, a key enzyme in dietary fat absorption.

Previous studies have reported that inhibiting pancreatic lipase increases fecal fat excretion (Tsujita et al., 2003; Tsujita and Takaku, 2009). Therefore, we compared the pancreatic lipase inhibition activity of BC with that of cellulose (Fig. 4). In our *in vitro* study using BA as an

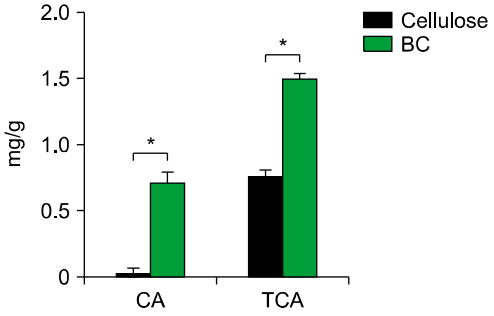

Fig. 3. Bile acid-binding capacity of cellulose and bamboo charcoal (BC). Data are presented as mean±SE (n=3). *P<0.05 (Student's t-test). CA, cholic acid; TCA, taurocholic acid.

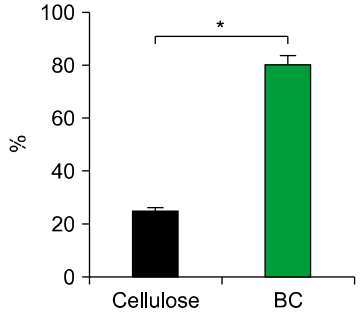

Fig. 4. Pancreatic lipase inhibition activity of cellulose and bamboo charcoal (BC). Data are presented as mean±SE (n=4). *P<0.05 (Student's t-test).

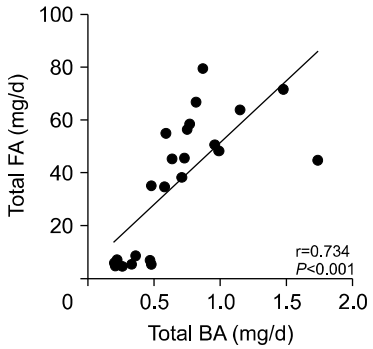

Fig. 5. Correlation between fecal total bile acid (BA) and fatty acid (FA) content. Data were evaluated using Pearson's correlation coefficient.

emulsifier, BC showed significantly higher pancreatic lipase inhibition activity than cellulose. Moreover, there was a positive correlation between total fecal BA and FA contents (Fig. 5). The emulsification of fat and lipase is essential for fat digestion. BC probably binds to BA in the gastrointestinal tract, inhibiting fat emulsification, possibly suppressing lipase activity.

A previous study reported that colipase-deficient mice experience overeating concurrent with massive fecal fat excretion (D'Agostino et al., 2002). However, we did not find compensatory overeating in the HF-BC group (Table 2). Therefore, the intake of ingredients that enhance fecal fat excretion potentially inhibits the absorption of fat-soluble vitamins (A, D, E, and K) besides fat. As for the dietary content of BC (0.5 wt%), no adverse effects seem to appear, as far as we could observe from the results of growth parameters, relative organ weights, and serum biochemical tests. However, increasing BC intake could result in adverse effects. Moreover, further toxicity studies should be conducted to determine the safe intake quantity of BC.

This study evaluated the growth parameters, relative organ weights, serum biochemical parameters, liver lipid content, and fecal lipid excretion of HFD-induced obese mice fed with BC for 80 days. The HFD containing BC reduced the relative epididymal and mesenteric WAT weights partly through enhanced fecal FA excretion compared with the HFD. Thus, dietary BC could be used as a food material to reduce visceral WAT weight.

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AUTHOR DISCLOSURE STATEMENT

KK and ST are employees of Kandagiko Co., Ltd., which supplied the bamboo charcoal. The other authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: KK, RH. Analysis and interpretation: KS, TS, KK, ST. Data collection: KS, TS, KK, ST. Writing the article: KS, RH, MY, KF. Critical revision of the article: KS, KK, RH, MY, KF. Final approval of the article: all authors. Statistical analysis: KS, RH, MY, KF. Obtained funding: KK, ST. Overall responsibility: KS, KK, RH.

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