Bioscience of Microbiota, Food and Health Vol. 43 (1), 13–22, 2024

Full Paper

Lactiplantibacillus plantarum 06CC2 upregulates intestinal ZO-1 protein and bile acid metabolism in Balb/c mice fed high-fat diet

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Received January 13, 2023; Accepted July 11, 2023; Published online in J-STAGE July 31, 2023

The effects of *Lactiplantibacillus plantarum* 06CC2 (LP06CC2), which was isolated from a Mongolian dairy product, on lipid metabolism and intestinal tight junction-related proteins in Balb/c mice fed a high-fat diet (HFD) were evaluated. The mice were fed the HFD for eight weeks, and the plasma and hepatic lipid parameters, as well as the intestinal tight junction–related factors, were evaluated. LP06CC2 slightly reduced the adipose tissue mass. Further, it dose-dependently decreased plasma total cholesterol (TC). The HFD tended to increase the plasma level of endotoxin and suppressed intestinal ZO-1 expression, whereas a low LP06CC2 dose increased ZO-1 expression and tended to reduce the plasma lipopolysaccharide level. Furthermore, a low LP06CC2 dose facilitated a moderate accumulation of Lactobacillales, a significant decrease in *Clostridium* cluster IV, and an increase in *Clostridium* cluster XVIII. The results obtained from analyzing the bile acids (BAs) in feces and cecum contents exhibited a decreasing trend for secondary and conjugated BAs in the low LP06CC2-dose group. Moreover, a high LP06CC2 dose caused excess accumulation of Lactobacillales and failed to increase intestinal ZO-1 and occludin expression, while the fecal butyrate level increased dose dependently in the LP06CC2-fed mice. Finally, an appropriate LP06CC2 dose protected the intestinal barrier function from the HFD and modulated BA metabolism.

Key words: *Lactiplantibacillus plantarum*, high-fat diet, secondary bile acid, intestinal barrier

INTRODUCTION

Obesity-related metabolic disorder is an emerging global health and economic challenge, and the application of food to prevent or mitigate it has been considered an effective measure. Recent studies on lactic acid bacteria (LAB) have afforded information on their preventive effects on metabolic disorders. Among them, a body fat-reducing effect was observed in studies utilizing *Lactiplantibacillus plantarum* (LP), previously called *Lactobacillus plantarum* [1]. Such an effect has been evaluated via animal studies and clinical trials with beneficial outcomes [2, 3]. The mechanism by which LAB exert their effects on body fat reduction is not fully understood and may differ depending on the type of strain and whether LAB are alive or dead. Protection of the intestinal tract barrier is among the plausible targets of LAB. Since intestinal tight junctions (TJs) are destabilized by obesity and a high-fat diet (HFD) [4], intestinal TJ destabilization is a key factor closely related to metabolic disorders that are associated with obesity, and this makes it a promising target for these diseases [5]. Intestinal TJs consist of assembly of several proteins, such as occludin, claudins, and ZO-1, and protect the intestinal mucosa from invasion by foreign substrates. Occludin and claudins are transmembrane proteins, and ZO-1 is a cytoplasmic protein containing protein-binding domains for claudins [6, 7]. Dietary components such as phytochemicals and dietary fibers are capable of enhancing TJ function by upregulating these assembly

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(Supplementary materials: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2480/)

doi: 10.12938/bmfh.2023-002

proteins [8, 9]. Moreover, many reports have indicated that LP stabilizes TJs, thereby implying that maintenance of intestinal barrier function is a promising mechanism [10–12]. When viable LAB are applied to experiments, it is valuable to focus on their metabolites. For example, LAB exhibit bile acid (BA) hydrolase activity and are expected to change the composition of intestinal BAs by deconjugation. BAs can modulate the stability of TJs depending on their molecular species [13–15]. We have previously considered the benefits of *L. plantarum* 06CC2 (LP06CC2), which was isolated from Mongolian dairy products and can alleviate influenza infection [16] and stimulate the induction of T helper type-1 cells [17]. More recently, we reported its ability to reduce hepatic cholesterol and decompose conjugated BAs in mice fed a high-cholesterol diet [18]. Here, we evaluated the effects of LP06CC2 on the expression of intestinal tight junction-related proteins and the BA composition of mice fed an HFD.

MATERIALS AND METHODS

Preparation of LP06CC2

As previously reported, LP06CC2 (a potential probiotic from Mongolian dairy products) can tolerate bile and gastric acids and adhere to Caco-2 cells [19]. LP06CC2 was precultured for 18 hr at 37°C in de Man, Rogosa, and Sharpe (MRS) broth (Merck Millipore, Darmstadt, Germany). Further, 5.0 mL of precultured suspension was inoculated into 500 mL of MRS broth and incubated for 18 hr at 37°C. After fermentation, the optical density was measured at a wavelength of 660 nm and determined to be approximately 5.0 to 5.5. The bacteria were harvested by centrifugation for 5 min at 1,500 × g, washed two times with phosphate-buffered saline (PBS), and lyophilized.

Mice and diet

The studies were conducted utilizing 5-week-old male Balb/c mice that were purchased from Japan SLC, Inc. (Shizuoka, Japan) and maintained at 22°C in a room with a 12-hr light–dark cycle. The mice were acclimatized for one week and divided into four groups (n=6 each). They were individually housed during experimental periods. They were fed an AIN-93G-based normal diet (ND) or HFD containing 0.1% or 1.0% LP06CC2 (LLB and HLB, respectively), as shown in Table 1, for eight weeks (from 6 to 14 weeks old). The experimental diets and water were fed *ad libitum*. During the feeding period, the diets were stored at −80°C, and colony forming units (cfu) were checked weekly to confirm that there was no decrease in viability. Body weights and food intake were recorded daily, and feces were collected daily for the final seven days of the experiment. The feces were pooled individually and preserved at −80°C. The animal studies were conducted according to the Guide for the Care and Use of Laboratory Animals of the University of Miyazaki (Animal Experiment Committee, University of Miyazaki; approval number: 2017-006) and in compliance with the Act on Welfare and Management of Animals (Japan; Act No. 105), Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Notice No. 88 of the Ministry of the Environment, Japan), and the Guidelines for Animal Experimentation (Japanese Association for Laboratory Animal Science). After the feeding period, blood samples were collected from the hearts of the mice under a triple anesthesia mix of 0.75 mg/kg medetomidine hydrochloride (Domitor; Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), 4.0 mg/kg midazolam (Dormicum; Astellas Pharma Inc., Tokyo, Japan), and 5.0 mg/kg butorphanol tartrate (Betorphal; Meiji Seika Pharma, Co., Ltd., Tokyo, Japan). The small and large intestines were excised, with the small intestine comprising the entire length from the pylorus to the ileocecal valve of the large intestine and the large intestine comprising the colon and rectum. The cecum was also excised, and its contents were lyophilized and subjected to BA analysis.

Plasma and hepatic lipids and lipopolysaccharide (LPS)

The liver and plasma triglyceride (TG) levels, as well as the total cholesterol (TC) levels, were measured by TG and cholesterol E-tests (Fujifilm Wako Pure Chemical Industries, Osaka, Japan), respectively. Before the analysis of hepatic lipids, the liver (100 mg) was homogenized (4,000 rpm for 3 min at 4°C) with a beads cell disruptor (Micro Smash MS-100R, Tomy Seiko Co., Ltd., Tokyo, Japan), and the lipids were extracted by the Folch method with a chloroform–methanol (2:1) solvent system [20]. Furthermore, the extract was dried in nitrogen gas and re-dissolved in 2-propanol containing 5% (v/v) Tween-20 to measure TG and TC. Plasma LPS was measured with a commercial kit (Limulus Color Test Wako, Fujifilm Wako) according to the manufacturer's protocol.

Table 1. Compositions of the experimental diets

Component (g/kg)	ND	HFD	HFD+LLB	HFD+HLB
Casein	200	200	200	200
Corn starch	529.5	199.5	198.5	179.5
Sucrose	100	100	100	100
Soybean oil	70	70	70	70
Lard	0	330	330	330
Cellulose	50	50	50	50
Choline bitartrate	2.5	2.5	2.5	2.5
L-cystine	3	3	3	3
AIN-93G Mineral mix	35	35	35	35
AIN-93 Vitamin mix	10	10	10	10
tert-Butylhydroquinone	0.0014	0.0014	0.0014	0.0014
LP06CC2	0	0	1	10
Total	1,000	1,000	1,000	1,000

ND: normal diet; HFD: high-fat diet; LLB: low dose lactic acid bacteria; HLB: high dose lactic acid bacteria.

doi: 10.12938/bmfh.2023-002

Western blotting

The large intestine was homogenized in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 50 mM NaF, 30 mM $Na_4P_2O_7$, and 2% (v/v) Triton X-100 together with a protease inhibitor cocktail (Nacalai Tesque, Inc., Kyoto, Japan) using an MS-100R beads cell disruptor with the abovementioned procedure for liver processing (Micro Smash MS-100R, Tomy Seiko Co., Ltd., Tokyo, Japan). The protein concentrations were determined with a bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), after which they were transferred to a poly (vinylidene fluoride) (PVDF) membrane. Subsequently, the membrane was blocked utilizing Blocking One-P (Nacalai Tesque, Inc.) for 30 min at room temperature (25°C). Next, the membrane was treated with antibodies recognizing ZO-1 (GTX88275, GeneTex, Irvine, CA, USA), occludin (OC-3F10, Thermo Fisher Scientific, Inc., Waltham, MA, USA), and β-actin (clone AC-15, Sigma, St. Louis, MO, USA). After the membrane was washed three times with Tris-buffered saline containing 0.1% Tween-20, it was treated with a secondary antibody for 1 hr at 25°C using anti-rabbit or anti-mouse IgG-HRP (Cell Signaling Technology, Danvers, MA, USA). Signals were visualized with an enhanced chemiluminescence (ECL) western blotting substrate (Bio-Rad, Hercules, CA, USA). Subsequently, the bands were quantified with a chemiluminescent imaging system (LAS-4000, Fujifilm, Tokyo, Japan). Furthermore, the intensities of the bands were normalized with a corresponding β-actin band as the internal control.

Liquid chromatography/mass spectrometry (LC/MS) analysis of BAs

BAs in the cecum contents and feces were measured by the method Hagio *et al.* [21]. The samples were lyophilized, after which 1 mL of ethanol was added to 100 mg of the ground samples to extract the BAs. Furthermore, nordeoxycholic acid (NDCA, 25 nmol) was added as an internal standard. The samples were sonicated and heated in a water bath for 30 min at 60°C. After cooling by immersion in cold running water, they were heated in boiling water for 3 min and centrifuged at 15°C for 10 min at 1,600 × g. Thereafter, the supernatants were collected. The precipitates were washed three times with ethanol and centrifuged for 1 min at 11,200 × g to collect the supernatant. The pooled extracts were evaporated and resolved in 1 mL of methanol, followed by purification with an Ultrafree-MC HV Centrifugal Filter unit before application to an LC/MS apparatus (MilliporeSigma, Burlington, MA, USA). The LC/MS analysis was performed on an Acquity UPLC H-class system (Waters, Milford, MA, USA), which was equipped with an Acquity UPLC HSS T3 column (1.8 µm, 100 mm × 2.1 mm; Waters), following a previous report [14]. Shortly, the mobile phase A was water containing 0.1% formic acid. The mobile phase B consisted of acetonitrile containing 0.1% formic acid. The solvents were eluted with a linearly increasing concentration gradient of acetonitrile at 0.4 mL/min. The acetonitrile concentrations were increased from 25% to 27%, 27% to 35%, 35% to 45%, 45% to 70%, and 70% to 100% for 4, 2, 9, 4, and 0.5 min, respectively. Electrospray ionization mass spectrometry (ESI-MS) was performed in negative ion mode, and the capillary voltage was adjusted to 3.2 kV.

LC/MS analysis of short-chain fatty acids

Short-chain fatty acids (SCFAs) were measured according to previous reports [18, 22, 23]. Fecal samples (150 mg) were suspended in 15 mL of water and centrifuged for 10 min at 8,000 × g, after which the supernatant was utilized for SCFA analysis. The supernatant (100 µL) was mixed with 100 µL of 500 µM 2-ethylbutyrate as the internal standard, 200 µL of 20 mM 2-nitrophenylhydrazine-HCl (2-NPH), and 400 µL of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-HCl. Thereafter, it was heated for 20 min at 60°C. Next, 100 µL of 15% potassium hydroxide in methanol-water (4:1) was added, and the mixture was heated for 15 min at 60°C. Furthermore, 2-NPH-labeled SCFAs were extracted two times with diethyl ether. Afterward, the extracts were evaporated and resolved in methanol for LC/MS analysis with an Acquity UPLC HSS T3 column (1.8 µm, 100 mm × 2.1 mm; Waters). Elution from the column was conducted with a linearly increasing concentration gradient of acetonitrile consisting of water containing 0.05% formic acid (mobile phase A) and acetonitrile containing 0.05% formic acid (mobile phase B) at a flow rate of 0.15 mL/min. The pump was programmed as follows: in the first 5 min, 20% mobile phase B, with mobile phase B subsequently increased from 20% to 60% for 15 min and then maintained for 2 min. A 10 µL portion of the sample was conditioned at 10°C and injected into the system within 8 hr. Next, the column temperature was maintained at 50°C. ESI-MS was performed in positive and negative ion mode, and the capillary voltage was adjusted to 3.0 kV.

Analysis of the intestinal microbiota

Deoxyribonucleic acid (DNA) extraction and terminal restriction fragment length polymorphism (T-RFLP) analyses were performed by TechnoSuruga Laboratory Co., Ltd. (Shizuoka, Japan) to evaluate the intestinal microbiota by the method of Nagashima *et al.* [24, 25]. Fecal samples were pretreated and then DNA was extracted, as previously reported [26]. DNA was purified utilizing a PI-480 automated DNA purification device equipped with a commercial kit (NR-201, Kurabo Industries, Osaka, Japan), following the manufacturer's protocol. Thereafter, 16S ribosomal DNA (rDNA) was amplified from the purified DNA utilizing the 6-carboxyfluorescein (6-FAM)-labeled 516F (5′-TGCCAGCAGCCGCGGTA-3) and 1510R (5′-GGTTACCTTGTTACGACTT-3) primers. The polymerase chain reaction (PCR) conditions were as follows: preheating at 95°C for 15 min; 30 cycles of a 3-step reaction comprising denaturation at 95°C for 30 sec, annealing at 50°C for 30 sec, and expansion at 72°C for 1 min; and then termination at 72°C for 10 min. The PCR products were purified and digested with FastDigest *Bse*LI (Bsll; Thermo Fisher Scientific, Inc., Waltham, MA, USA) for 10 min at 37°C. Furthermore, the T-RFLP peaks of each sample were assigned to operational taxonomic units (OTUs), and taxonomic groups were estimated based on the human intestinal flora database (https://www.tecsrg.co.jp/services/community-structure-analysis/). The taxa, which were included in the order Clostridiales, are shown separately for each *Clostridium* cluster. OTUs without an estimated taxon or more than one estimated taxon were classified as "others". Alpha diversity was calculated using the Shannon–Wiener index [27].

Statistical analyses

Data are presented as the mean ± SD. Statistical analyses were performed using Statistical Analysis for Mac, Version. 3.0 (Esumi Co., Ltd, Tokyo, Japan). A post hoc test was performed after a one-way ANOVA. The Tukey–Kramer test was performed to evaluate significant differences among the dietary groups when significant interaction ($p<0.05$) was detected.

RESULTS

Growth parameters

Table 2 presents the body weight and food intake data. The food intakes of the HFD, LLB, and HLB groups were lower than those of the ND group, although LP06CC2 did not exert any effect on food intake. The HFD slightly increased body weights, whereas LP06CC2 did not affect body weights (Table 2). The total calorie intakes of the dietary groups were comparable. Successive changes in body weight and cumulative food intake are shown in Supplementary Fig. 1A and 1B, respectively. Table 3 shows the weights of the tissues and shows that the HFD and LP06CC2 did not affect the weights of the liver and small and large intestines. Regarding the HFD group, the weights of epididymal and retroperitoneal adipose tissues significantly increased compared with those of the ND group. Moreover, these significant differences were not detected between the ND and LLB groups. The total weight of the adipose tissues (epididymal + perirenal + retroperitoneal tissues) was suppressed by 11.2% in the LLB group and 8.4% in the HLB group.

Lipid parameters

The results of analysis of the plasma and liver lipid profiles are presented in Table 4. Hepatic TG was significantly higher in the HFD group than in the ND group. LP06CC2 did not affect the hepatic TG and TC levels at any dose. However, plasma TC was moderately but not significantly increased in the HFD group, and the level in the HLB group was significantly lower than that in the HFD group. Although it was not significant, plasma TG tended to decrease in the LLB and HLB groups.

Intestinal TJ-related parameters

Next, we evaluated the plasma LPS level and protein expression of ZO-1 and occludin in the large intestine (Fig. 1). The plasma LPS levels of the HFD mice were slightly but not significantly higher than those of the ND mice (Fig. 1A). LP06CC2 administration led to a tendency for the plasma LPS level to be low as compared with the HFD group. As structural

Table 2. Body weight and food intake

	ND	HFD	HFD+LLB	HFD+HLB
Final body weight	27.5 ± 0.6	29.7 ± 0.5	29.8 ± 1.0	30.8 ± 0.7
Food intake (g/2-day)	7.5 ± 0.4^{a}	4.9 ± 0.1^{b}	4.8 ± 0.1^{b}	4.8 ± 0.1^{b}
Calorie intake (kcal/day)	32.6 ± 1.9	29.2 ± 0.5	28.8 ± 0.7	32.2 ± 2.8

Data are means ± SD for 6 mice. $^{a, b}$Values without any common alphabetic letter are significantly different each other at $p<0.05$. ND: normal diet; HFD: high-fat diet; LLB: low dose lactic acid bacteria; HLB: high dose lactic acid bacteria; SD: standard deviation.

Table 3. Tissue weights

(mg/g body weight)	ND	HFD	HFD+LLB	HFD+HLB
Liver	39.2 ± 1.7	38.7 ± 1.0	40.1 ± 0.8	42.4 ± 1.1
Small intestine	29.7 ± 0.7	30.4 ± 0.5	29.1 ± 0.8	31.2 ± 0.9
Large intestine	5.4 ± 0.2	5.4 ± 0.5	5.0 ± 0.2	5.0 ± 0.3
Epididymal fat	15.8 ± 1.5^{a}	26.6 ± 1.5^{b}	24 ± 3.0^{ab}	24.4 ± 2.1^{b}
Perirenal fat	2.8 ± 0.3	3.4 ± 0.3	3.1 ± 0.4	3.4 ± 0.4
Retroperitoneal fat	4.3 ± 0.5^{a}	7.9 ± 0.4^{b}	6.5 ± 0.7^{ab}	6.5 ± 0.6^{ab}

Data are means ± SD for 6 mice. $^{a, b}$Values without any common alphabetic letter are significantly different each other at $p<0.05$. ND: normal diet; HFD: high-fat diet; LLB: low dose lactic acid bacteria; HLB: high dose lactic acid bacteria; SD: standard deviation.

Table 4. Plasma and hepatic lipid parameters

	ND	HFD	HFD+LLB	HFD+HLB
Liver (mg/g)				
Total cholesterol	10.3 ± 1.0	12.6 ± 0.9	12.4 ± 0.6	13.3 ± 1.0
Triglycerides	48.3 ± 4.7^{a}	87.5 ± 5.9^{b}	88.8 ± 4.3^{b}	91.3 ± 14.9^{b}
Plasma (mg/dL)				
Total cholesterol	97.5 ± 7.7^{ab}	121.1 ± 12.0^{b}	97.5 ± 7.0^{ab}	83.6 ± 6.5^{a}
Triglycerides	132.8 ± 9.3	130.6 ± 12.5	102.7 ± 7.9	93.6 ± 12.6

Data are means ± SD for 6 mice. $^{a, b}$Values without any common alphabetic letter are significantly different each other at $p<0.05$. ND: normal diet; HFD: high-fat diet; LLB: low dose lactic acid bacteria; HLB: high dose lactic acid bacteria SD: standard deviation.

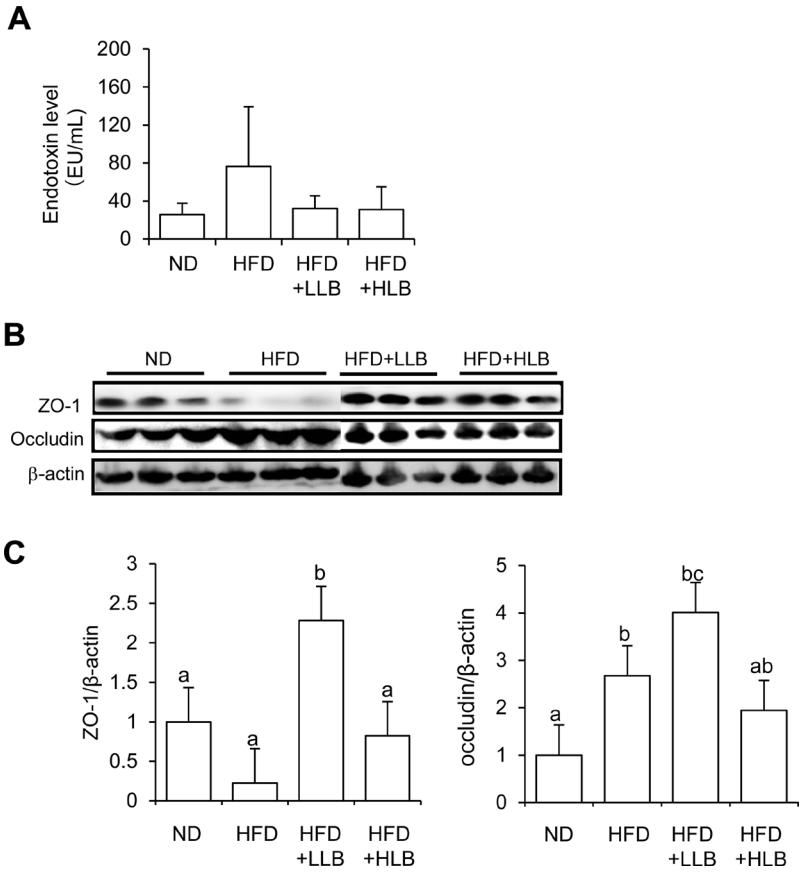

Fig. 1. Effect of *Lactiplantibacillus plantarum* 06CC2 on intestinal barrier–related parameters. Plasma LPS level was evaluated by the Limulus test (A). Data are mean ± SD values for 6 mice. ZO-1 and occludin proteins were detected by western blot (B), and expression intensity was standardized by the expression of β-actin (C). [a–c]Values without letters in common are significantly different from each other at $p<0.05$.

ND: normal diet; HFD: high-fat diet; LLB: low dose lactic acid bacteria; HLB: high dose lactic acid bacteria; SD: standard deviation.

proteins of TJs, ZO-1 and occludin were detected by the western blot analysis (Fig. 1B and 1C). Compared with the ND group, ZO-1 expression was moderately decreased in the HFD group ($p=0.052$), whereas that of occludin was increased. The LLB diet significantly increased ZO-1 expression compared with the HFD, whereas the HLB diet did not. Occludin expression was also the highest in the LLB group, and significant differences were observed between the ND and LLB groups.

BA analysis

The results of analysis of the cecum and fecal contents of BAs are shown in Figs. 2 and 3, respectively. Deoxycholate (DCA), lithocholate (LCA), and hyodeoxycholate (HDCA) were detected as secondary BAs in both the cecum and fecal contents. The HFD significantly increased the cecum total BA level, which was slightly suppressed in the LLB and HLB groups. The HFD slightly, but not significantly, increased the cecum DCA and total secondary BA levels (Fig. 2). The cecum DCA and total secondary BA levels were significantly increased in the HLB group compared with the ND group. The HFD did not change the total secondary BA level, whereas the level was significantly increased in the HLB group compared with the ND group (Fig. 2). The LLB diet slightly, but not significantly, decreased the fecal DCA, LCA, HDCA, and total secondary BA levels compared with the HFD (Fig. 3). The total fecal-conjugated BA level increased significantly in the HFD group, and the same trend was also observed in the cecum content ($p=0.064$; the detailed compositions of BAs are shown in the Supplementary Fig. 2). The LLB diet partially abolished the increase in the total fecal-conjugated BA levels.

Fecal SCFA analyses

The results of measurement of the fecal SCFA levels by LC/MS are shown in Fig. 4. The HFD significantly decreased the *iso*-valerate level and tended to decrease the propionate and *n*-butyrate levels. LP06CC2 dose-dependently increased the fecal *n*-butyrate and *iso*-valerate levels, and significant differences were observed between the HFD and HLB groups. Acetate, *iso*-butyrate, and *n*-valerate were comparable among the dietary groups. The total SCFA levels, calculated as the sum of SCFAs in Fig. 4, were comparable among the dietary groups. Lactate is not an SCFA, but it was dose-dependently increased by LP06CC2.

Analysis of the intestinal microbiota

To estimate intestinal microbiota, fecal samples were analyzed by the T-RFLP method (Table 5). The HFD slightly, but not significantly, reduced Lactobacillales, whereas the LLB diet tended to ameliorate this decrease. The HLB diet caused an approximately 8.5-fold increase in Lactobacillales compared with the HFD. The HFD significantly decreased *Bacteroides*

doi: 10.12938/bmfh.2023-002

and increased *Prevotella*. The *Prevotella* levels were strikingly decreased in the HLB group compared with the HFD group. *Clostridium* cluster IV was slightly increased in the HFD group, whereas it was not detected in the LLB and HLB groups. *Clostridium* subcluster XIVa was slightly decreased in the HFD and LLB groups, whereas the HLB diet had no apparent effect.

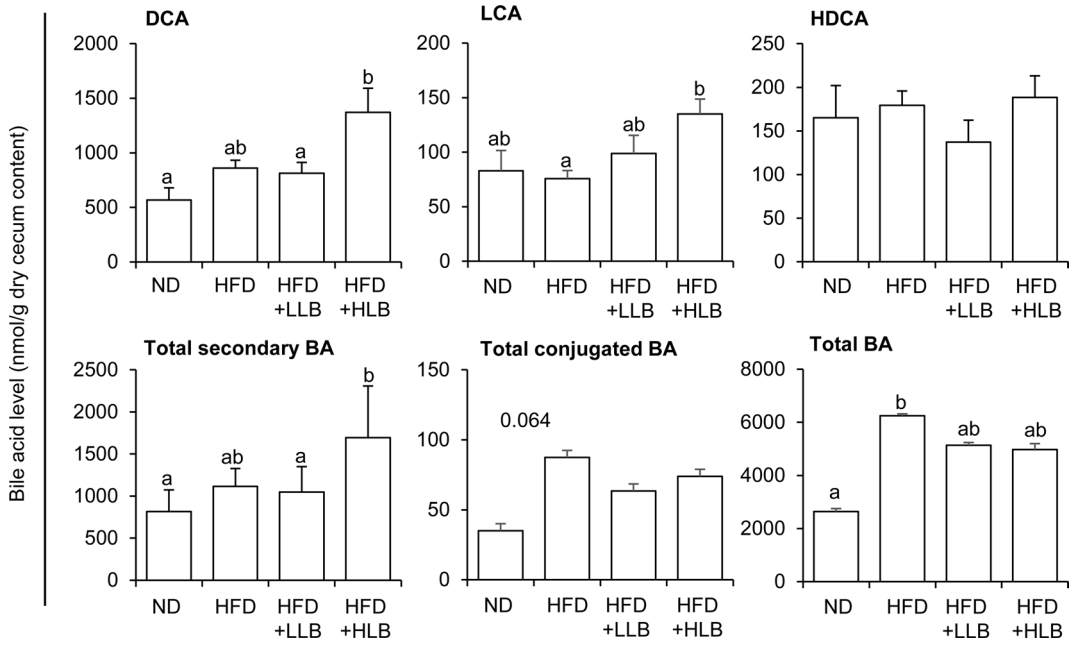

Fig. 2. Effect of *Lactiplantibacillus plantarum* 06CC2 on the bile acid composition in cecum contents. Bile acids were measured by the LC/MS method. Data are mean ± SD values for 6 mice. Total secondary BAs include DCA, LCA, and HDCA. Total conjugated BAs include taurine-conjugated cholate, chenodeoxycholate, DCA, LCA, HDCA, α-muricholate, and β-muricholate. BAs, bile acids; DCA, deoxycholate; LCA, lithocholate; HDCA, hyodeoxycholate. [a, b]Values without letters in common are significantly different from each other at $p<0.05$.

ND: normal diet; HFD: high-fat diet; LLB: low dose lactic acid bacteria; HLB: high dose lactic acid bacteria; SD: standard deviation.

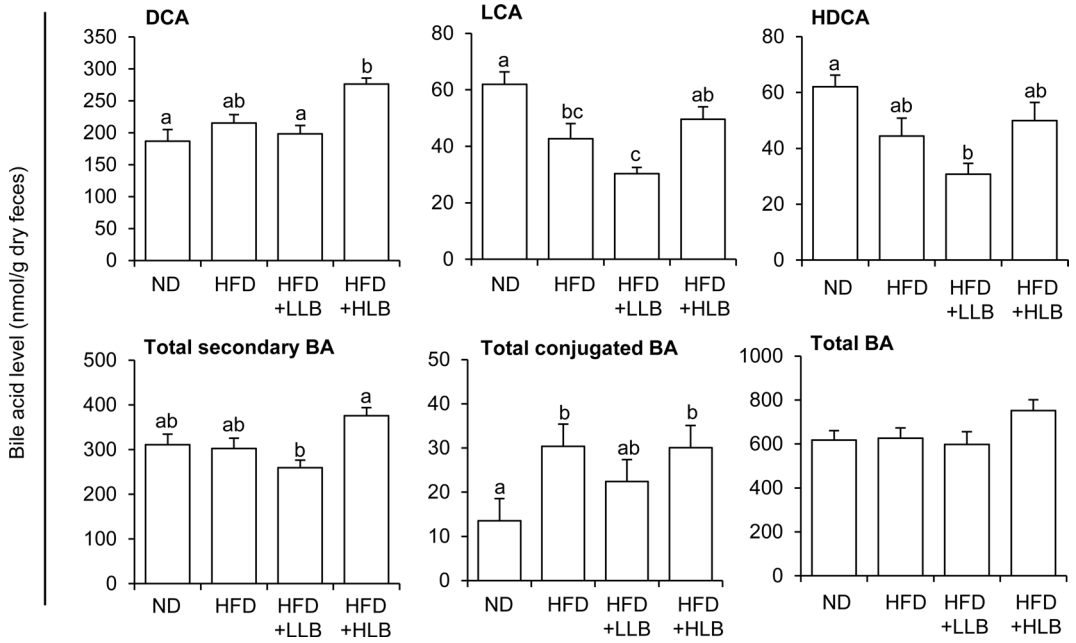

Fig. 3. Effect of *Lactiplantibacillus plantarum* 06CC2 on the bile acid composition in feces. Bile acids were measured by the LC/MS method. Data are mean ± SD values for 6 mice. Total secondary BAs include DCA, LCA, and HDCA. Total conjugated BAs include taurine-conjugated cholate, chenodeoxycholate, DCA, LCA, HDCA, α-muricholate, and β-muricholate. BAs, bile acids; DCA, deoxycholate; LCA, lithocholate; HDCA, hyodeoxycholate. [a–c]Values without letter in common are significantly different from each other at $p<0.05$.

ND: normal diet; HFD: high-fat diet; LLB: low dose lactic acid bacteria; HLB: high dose lactic acid bacteria; SD: standard deviation.

doi: 10.12938/bmfh.2023-002

With regards to the Shannon index, the LLB diet significantly increased the alpha diversity compared with the HFD (Fig. 5).

DISCUSSION

The body fat-reducing effect of LP has been demonstrated by different strains, and the data in this study also suggest that LP06CC2 can moderately reduce body fat increased by HFD consumption (Table 3). Intriguingly, the data did not reveal a dose-dependent effect, and a low LP06CC2 dose efficiently reduced the masses of the adipose tissues. Here, we added 0.1% and 1.0% lyophilized LP06CC2, which corresponded to 3.9×10^8 and 3.9×10^9 cfu/g diet, to the LLB and HLB diets, respectively. Therefore, based on the estimated food intakes, the estimated daily intake of LP06CC2 was 9.4×10^8 and 9.4×10^9 cfu in the LLB and HLB groups, respectively. Many studies have applied 1×10^8–1×10^9

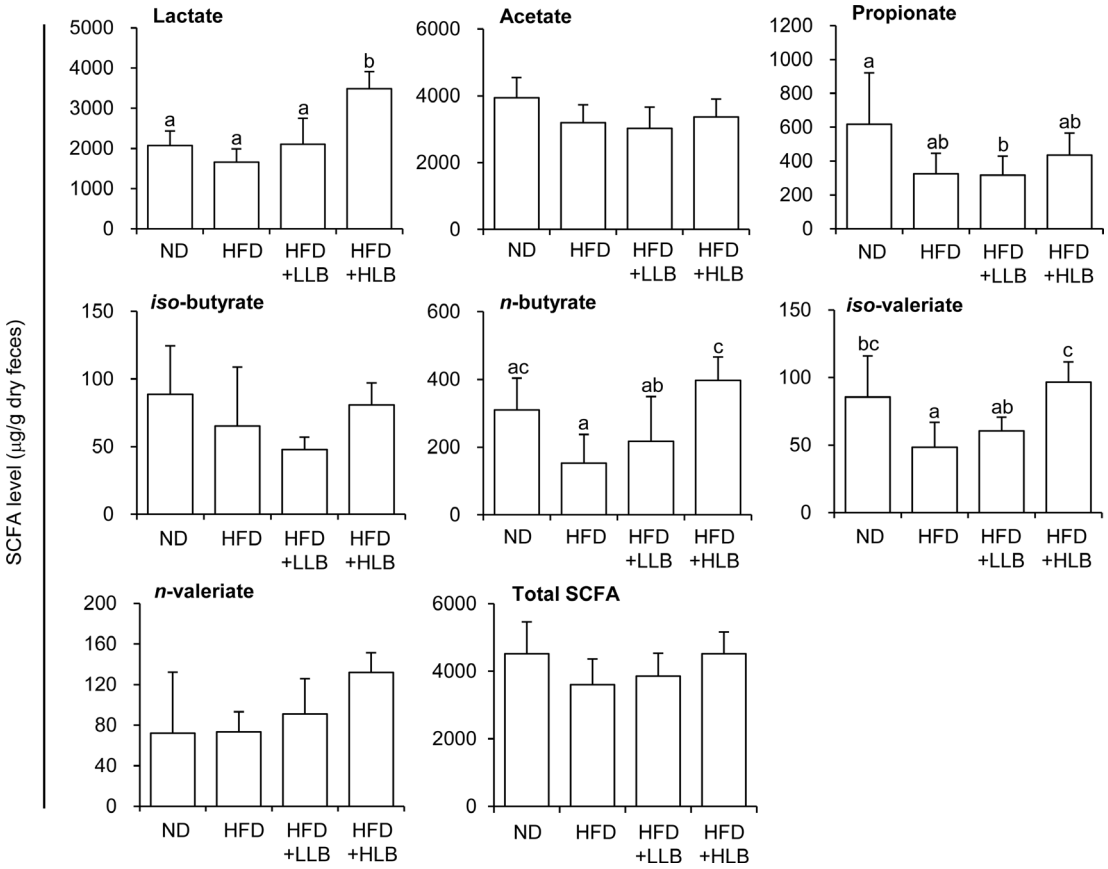

Fig. 4. Effect of *Lactiplantibacillus plantarum* 06CC2 on the short-chain fatty acid composition in feces. Data are mean ± SD values for 6 mice. Short-chain fatty acids were measured by the LC/MS method. Total SCFAs include all fatty acids shown in this figure except for lactate. SCFAs, short-chain fatty acids. [a–c]Values without letters in common are significantly different from each other at $p<0.05$.

ND: normal diet; HFD: high-fat diet; LLB: low dose lactic acid bacteria; HLB: high dose lactic acid bacteria; SD: standard deviation.

Table 5. Analysis of the intestinal microbiota by T-RFLP

	ND	HFD	HFD+LLB	HFD+HLB
	Occupation ratio of phylogenetic groups (%)			
Bifidobacterium	nd	0.1 ± 0.1	nd	nd
Lactobacillales	11.2 ± 4.7[a]	6.9 ± 0.9[a]	17.5 ± 1.9[a]	58.9 ± 4.1[b]
Bacteroides	42.2 ± 5.9[b]	19.4 ± 9.0[a]	12.7 ± 1.8[a]	11.6 ± 2.9[a]
Prevotella	17.2 ± 6.6[ab]	52.0 ± 12.8[c]	43.2 ± 4.2[bc]	8.5 ± 2.0[a]
Clostridium cluster IV	0.2 ± 0.1[ab]	0.4 ± 0.2[b]	nd[a]	nd[a]
Clostridium subcluster XIVa	22.5 ± 3.5	13.0 ± 4.9	15.4 ± 2.4	14.0 ± 0.9
Clostridium cluster XI	0.4 ± 0.2	nd	nd	nd
Clostridium cluster XVIII	1.6 ± 0.5[a]	2.8 ± 0.5[ab]	4.3 ± 0.2[b]	2.0 ± 0.3[a]
others	4.8 ± 0.4	5.3 ± 0.4	7.0 ± 0.2	5.0 ± 0.3

Data are means ± SD for 6 mice. [a–c]Values without any common alphabetic letter are significantly different each other at $p<0.05$. T-RFLP: Terminal Restriction Fragment Length Polymorphism; ND: normal diet; HFD: high-fat diet; LLB: low dose lactic acid bacteria; HLB: high dose lactic acid bacteria; nd: not detected; SD: standard deviation.

doi: 10.12938/bmfh.2023-002

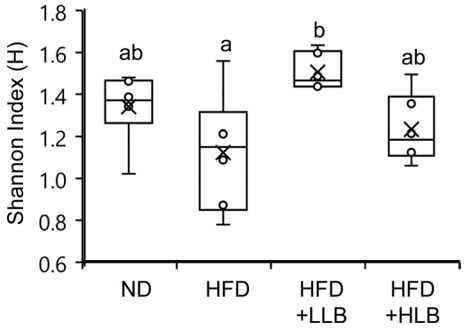

Fig. 5. Alpha diversity of intestinal microbiota evaluated by Shannon index. Data are mean ± SD values for 6 mice. [a, b]Values without letters in common are significantly different from each other at $p<0.05$.

ND: normal diet; HFD: high-fat diet; LLB: low dose lactic acid bacteria; HLB: high dose lactic acid bacteria SD: standard deviation.

cfu/day of various strains of LP to effectively reduce the mass of adipose tissue [28–33]. Therefore, the ability of LP06CC2 to reduce body fat was assumed to be almost comparable with those of other strains possessing body-fat reducing effects.

Notably, the plasma cholesterol levels were dose-dependently reduced by LP06CC2. Our previous study indicated that LP06CC2 could reduce hepatic cholesterol by promoting the deconjugation of BAs in hypercholesterolemic mice induced by a cholesterol- and BA-containing diet [18]. In this previous study, a reduction in hepatic cholesterol was not observed in mice fed a normal diet, and no apparent effect of LP06CC2 was observed on hepatic cholesterol. As the HFD in the present study did not induce apparent accumulation of hepatic cholesterol (Table 4), it is assumed that the effects of LP06CC2 are clearly observed under conditions of abnormal BA and cholesterol metabolism. In our previous study, direct deconjugation of taurocholic acid into cholic acid by LP06CC2 was observed. The moderate reduction of total conjugated BAs in the feces and cecum contents in the present study, as shown in Figs. 2 and 3, might have been caused by the BA hydrolase reaction. Conversely, the total conjugated BA levels of the HFD and HLB groups were comparable, irrespective of the significant reduction in plasma cholesterol.

Intestinal barrier function is a key to maintaining the whole-body metabolic pathway, and intake of an HFD can deteriorate TJs, thus destabilizing intestinal barrier function, a reduction in structural TJ proteins and an increase in plasma LPS levels could reflect such destabilization [5, 34–37]. The influx of LPS from the intestinal tract to the adipose tissue facilitated an inflammatory response and expansion of the adipose tissue [38]. Therefore, it was rational that the tendency for plasma LPS to be reduced by LP06CC2 contributed to the reduction in the adipose tissue mass. Stabilization of the intestinal barrier accompanied by a reduction in the adipose tissue mass was observed with the administration of an LPKC28 strain [39]. Here, a moderate increase in plasma LPS and a reduction in the intestinal ZO-1 levels were observed in the HFD group compared with the ND group. However, the effect of an HFD on the intestinal occludin level has been controversial [15, 34, 40, 41]. The data in this study were consistent with those of several studies in which the deterioration of intestinal barrier function was observed as a result of intake of an HFD but without the suppression of occludin expression. LP06CC2, especially a low dose, recovered the ZO-1 expression and suppressed the HFD-induced elevation of plasma LPS, indicating the protection of intestinal barrier function from the effects of intake of an HFD. Since LP06CC2 increased the fecal butyrate levels, butyrate might be one of the plausible factors that facilitated the stabilization of TJs by LP06CC2 [42–44]. Conversely, the increase in butyrate depended on the LP06CC2 dose, whereas its effect on TJs was not dose dependent, although it was potent at a low dose. Therefore, an increase in butyrate could not sufficiently explain the stabilization of TJs induced by LP06CC2. We previously reported that an increase in fecal butyrate as a result of intake of LP06CC2 was strongly correlated with the fecal lactate level [18]. Correlative analyses of the fecal lactate and butyrate levels using the present data demonstrated a significant ($p<0.001$) positive correlation, which validated the use of lactate as a substrate for the synthesis of butyrate [45–47]. Lactic acid is one of the representative metabolites produced from LAB, and the fecal lactate level was in fact increased in the HLB group. Several recent studies have shown that lactic acid was capable of promoting the integrity of epithelial barrier function. For instance, Delgado-Diaz *et al.* revealed efficacy with respect to cervicovaginal epithelial barrier integrity [48], and Huang *et al.* revealed that lactate provided protection against gastric mucosal injury and the inflammatory response induced by ethanol administration by upregulating TJ proteins [49]. On the other hand, it is unlikely that lactic acid contributed to the function of LP06CC2 in the present study, as the LLB diet promoted ZO-1 expression but elevation of fecal lactic acid was not observed.

The reduction of conjugated BA by LP06CC2 could be explained by its BA hydrolase activity [18], whereas 7-α dehydroxylation of primary BAs by several specific microbiotas accounted for the production of the secondary BAs. In previous studies, *Clostridium* subcluster XIVa was present in human fecal and intestinal microbiotas, which could mediate 7-α dehydroxylation to produce secondary BAs [50, 51]. Here, although *Clostridium* subcluster XIVa was slightly increased by the low dose of LP06CC2, the HFD decreased in abundance even though no effect was observed on the secondary fecal BA level. Therefore, a subdivided analysis at the species level is required to reveal the detailed microbiota and discuss the involvement of microbiota manipulation in the stabilization of TJs by LP06CC2. Although the occupancy of Lactobacillales in the HLB group was over five times higher than in the ND group, there was no significant difference between the LLB and ND groups. As this study was conducted using live bacteria, it was estimated

that LP06CC2 was able to reach the intestinal tract as a viable bacterium due to its probiotic property [19]. On the other hand, as T-RFLP analysis revealed the occupation ratios of phylogenetic groups, we could not confirm the contribution of LP06CC2 toward the increase of Lactobacillales in the HLB group. Although there are several articles in which beneficial health effects of LP06CC2 have been shown, our previous article [18] is the only previous animal study using live LP06CC2. Therefore, attention should be paid to the comparison of data interpretations between the present data and those in studies using dead bacteria.

The Shannon diversity index is one of the most commonly used indices for alpha diversity and is higher when the number of species is high and each species is equally present. A high-fat diet was shown to decrease the alpha diversity of intestinal microbiota as evaluated by the Shannon diversity index [52]. Figure 5 shows a significant increase in the Shannon diversity index in the LLB group compared with the HFD group, indicating an increase of alpha diversity in the LLB group. Since alleviation of the HFD-induced reduction in Shannon diversity index was no longer observed in the HLB group and the Lactobacillales level of the HLB group was abnormally increased, the estimated HLB dose was too high to be taken daily by humans. Therefore, the dose for the LLB diet was considered to be very appropriate for protection of the intestinal barrier and regulation of intestinal bacteria.

In conclusion, the present data indicate that LP06CC2 has the ability to stabilize the intestinal barrier in mice fed a high-fat diet, leading to suppression of body fat mass. In addition, to accomplish this effect, there might be an appropriate dose of LP06CC2. Recently, the protective effect of LP06CC2 against inflammatory bowel disease via the promotion of interleukin (IL)-10 production was reported [53]. IL-10 is anti-inflammatory cytokine and has a protective effect on intestinal barrier function [54]. Further studies are needed to elucidate the correlation between intestinal anti-inflammatory effects and the prevention of metabolic disorders.

CONFLICTS OF INTEREST

We declare no conflicts of interest associated with this manuscript.

REFERENCES

1. Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, O'Toole PW, Pot B, Vandamme P, Walter J, Watanabe K, Wuyts S, Felis GE, Gänzle MG, Lebeer S. 2020. A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. Int J Syst Evol Microbiol 70: 2782–2858. [Medline] [CrossRef]
2. Crovesy L, Ostrowski M, Ferreira DMTP, Rosado EL, Soares-Mota M. 2017. Effect of *Lactobacillus* on body weight and body fat in overweight subjects: a systematic review of randomized controlled clinical trials. Int J Obes 41: 1607–1614. [Medline] [CrossRef]
3. Huang WC, Lee MC, Lee CC, Ng KS, Hsu YJ, Tsai TY, Young SL, Lin JS, Huang CC. 2019. Effect of *Lactobacillus plantarum* TWK10 on exercise physiological adaptation, performance, and body composition in healthy humans. Nutrients 11: 2836. [Medline] [CrossRef]
4. Massier L, Blüher M, Kovacs P, Chakaroun RM. 2021. Impaired intestinal barrier and tissue bacteria: pathomechanisms for metabolic diseases. Front Endocrinol (Lausanne) 12: 616506. [Medline] [CrossRef]
5. Rohr MW, Narasimhulu CA, Rudeski-Rohr TA, Parthasarathy S. 2020. Negative effects of a high-fat diet on intestinal permeability: a review. Adv Nutr 11: 77–91. [Medline] [CrossRef]
6. Furuse M, Itoh M, Hirase T, Nagafuchi A, Yonemura S, Tsukita S, Tsukita S. 1994. Direct association of occludin with ZO-1 and its possible involvement in the localization of occludin at tight junctions. J Cell Biol 127: 1617–1626. [Medline] [CrossRef]
7. Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S. 1998. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Biol 141: 1539–1550. [Medline] [CrossRef]
8. Suzuki T. 2020. Regulation of the intestinal barrier by nutrients: the role of tight junctions. Anim Sci J 91: e13357. [Medline] [CrossRef]
9. Lee B, Moon KM, Kim CY. 2018. Tight junction in the intestinal epithelium: its association with diseases and regulation by phytochemicals. J Immunol Res 2018: 2645465. [Medline] [CrossRef]
10. Tang T, Song J, Li J, Wang H, Zhang Y, Suo H. 2020. A synbiotic consisting of *Lactobacillus plantarum* S58 and hull-less barley β-glucan ameliorates lipid accumulation in mice fed with a high-fat diet by activating AMPK signaling and modulating the gut microbiota. Carbohydr Polym 243: 116398. [Medline] [CrossRef]
11. Karczewski J, Troost FJ, Konings I, Dekker J, Kleerebezem M, Brummer RJ, Wells JM. 2010. Regulation of human epithelial tight junction proteins by *Lactobacillus plantarum in vivo* and protective effects on the epithelial barrier. Am J Physiol Gastrointest Liver Physiol 298: G851–G859. [Medline] [CrossRef]
12. Wang J, Ji H, Wang S, Liu H, Zhang W, Zhang D, Wang Y. 2018. Probiotic *Lactobacillus plantarum* promotes intestinal barrier function by strengthening the epithelium and modulating gut microbiota. Front Microbiol 9: 1953. [Medline] [CrossRef]
13. Raimondi F, Santoro P, Barone MV, Pappacoda S, Barretta ML, Nanayakkara M, Apicella C, Capasso L, Paludetto R. 2008. Bile acids modulate tight junction structure and barrier function of Caco-2 monolayers via EGFR activation. Am J Physiol Gastrointest Liver Physiol 294: G906–G913. [Medline] [CrossRef]
14. Sarathy J, Detloff SJ, Ao M, Khan N, French S, Sirajuddin H, Nair T, Rao MC. 2017. The Yin and Yang of bile acid action on tight junctions in a model colonic epithelium. Physiol Rep 5: e13294. [Medline] [CrossRef]
15. Ahmad R, Rah B, Bastola D, Dhawan P, Singh AB. 2017. Singh obesity-induces organ and tissue specific tight junction restructuring and barrier deregulation by claudin switching. Sci Rep 7: 5125. [Medline] [CrossRef]
16. Takeda S, Takeshita M, Kikuchi Y, Dashnyam B, Kawahara S, Yoshida H, Watanabe W, Muguruma M, Kurokawa M. 2011. Efficacy of oral administration of heat-killed probiotics from Mongolian dairy products against influenza infection in mice: alleviation of influenza infection by its immunomodulatory activity through intestinal immunity. Int Immunopharmacol 11: 1976–1983. [Medline] [CrossRef]
17. Takeda S, Kawahara S, Hidaka M, Yoshida H, Watanabe W, Takeshita M, Kikuchi Y, Bumbein D, Muguruma M, Kurokawa M. 2013. Effects of oral administration of probiotics from Mongolian dairy products on the Th1 immune response in mice. Biosci Biotechnol Biochem 77: 1372–1378. [Medline] [CrossRef]
18. Yamasaki M, Minesaki M, Iwakiri A, Miyamoto Y, Ogawa K, Nishiyama K, Tsend-Ayush C, Oyunsuren T, Li Y, Nakano T, Takeshita M, Arima Y. 2020. *Lactobacillus plantarum* 06CC2 reduces hepatic cholesterol levels and modulates bile acid deconjugation in Balb/c mice fed a high-cholesterol diet. Food Sci Nutr 8: 6164–6173. [Medline] [CrossRef]
19. Takeda S, Yamasaki K, Takeshita M, Kikuchi Y, Tsend-Ayush C, Dashnyam B, Ahhmed AM, Kawahara S, Muguruma M. 2011. The investigation of probiotic potential of lactic acid bacteria isolated from traditional Mongolian dairy products. Anim Sci J 82: 571–579. [Medline] [CrossRef]
20. Folch J, Lees M, Sloane Stanley GH. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226: 497–509. [Medline] [CrossRef]
21. Hagio M, Matsumoto M, Fukushima M, Hara H, Ishizuka S. 2009. Improved analysis of bile acids in tissues and intestinal contents of rats using LC/ESI-MS. J Lipid Res 50: 173–180. [Medline] [CrossRef]
22. Inoue H, Takayama K, Takahara C, Tabuchi N, Okamura N, Narahara N, Kojima E, Date Y, Tsuruta Y. 2019. Determination of short-chain fatty acids in mouse feces by high-performance liquid chromatography using 2-nitrophenylhydrazine as a labeling reagent. Biol Pharm Bull 42: 845–849. [Medline] [CrossRef]
23. Miwa H, Yamamoto M, Nishida T, Nunoi K, Kikuchi M. 1987. High-performance liquid chromatographic analysis of serum long-chain fatty acids by direct derivatization method. J Chromatogr A 416: 237–245. [Medline] [CrossRef]
24. Nagashima K, Hisada T, Sato M, Mochizuki J. 2003. Application of new primer-enzyme combinations to terminal restriction fragment length polymorphism profiling of bacterial populations in human feces. Appl Environ Microbiol 69: 1251–1262. [Medline] [CrossRef]
25. Nagashima K, Mochizuki J, Hisada T, Suzuki S, Shimomura K. 2006. P2006. hylogenetic analysis of 16S ribosomal RNA gene sequences from human fecal microbiota and improved utility of terminal restriction fragment length polymorphism profiling. Biosci Microflora 25: 99–107. [CrossRef]
26. Takahashi S, Tomita J, Nishioka K, Hisada T, Nishijima M. 2014. Development of a prokaryotic universal primer for simultaneous analysis of *Bacteria* and *Archaea* using next-generation sequencing. PLoS One 9: e105592. [Medline] [CrossRef]
27. Shannon CE. 1948. A mathematical theory of communication. Bell Syst Tech J 27: 379–423. [CrossRef]
28. Okubo T, Takemura N, Yoshida A, Sonoyama K. 2013. KK/Ta Mice administered *Lactobacillus plantarum* Strain No. 14 have lower adiposity and higher insulin

sensitivity. Biosci Microbiota Food Health 32: 93–100. [Medline] [CrossRef]
29. Sakai T, Taki T, Nakamoto A, Shuto E, Tsutsumi R, Toshimitsu T, Makino S, Ikegami S. 2013. *Lactobacillus plantarum* OLL2712 regulates glucose metabolism in C57BL/6 mice fed a high-fat diet. J Nutr Sci Vitaminol (Tokyo) 59: 144–147. [Medline] [CrossRef]
30. Choi WJ, Dong HJ, Jeong HU, Ryu DW, Song SM, Kim YR, Jung HH, Kim TH, Kim YH. 2020. *Lactobacillus plantarum* LMT1-48 exerts anti-obesity effect in high-fat diet-induced obese mice by regulating expression of lipogenic genes. Sci Rep 10: 869. [Medline] [CrossRef]
31. Takemura N, Okubo T, Sonoyama K. 2010. *Lactobacillus plantarum* strain No. 14 reduces adipocyte size in mice fed high-fat diet. Exp Biol Med (Maywood) 235: 849–856. [Medline] [CrossRef]
32. Wu CC, Weng WL, Lai WL, Tsai HP, Liu WH, Lee MH, Tsai YC. 2015. Effect of *Lactobacillus plantarum* strain K21 on high-fat diet-fed obese mice. Evid Based Complement Alternat Med 2015: 391767. [Medline] [CrossRef]
33. Cai H, Wen Z, Li X, Meng K, Yang P. 2020. *Lactobacillus plantarum* FRT10 alleviated high-fat diet-induced obesity in mice through regulating the PPARα signal pathway and gut microbiota. Appl Microbiol Biotechnol 104: 5959–5972. [Medline] [CrossRef]
34. Suzuki T, Hara H. 2010. Dietary fat and bile juice, but not obesity, are responsible for the increase in small intestinal permeability induced through the suppression of tight junction protein expression in LETO and OLETF rats. Nutr Metab (Lond) 7: 19. [Medline] [CrossRef]
35. Moreira AP, Texeira TF, Ferreira AB, Peluzio MC, Alfenas RC. 2012. Influence of a high-fat diet on gut microbiota, intestinal permeability and metabolic endotoxaemia. Br J Nutr 108: 801–809. [Medline] [CrossRef]
36. Mujawdiya PK, Sharma P, Sharad S, Kapur S. 2020. Reversal of increase in intestinal permeability by *Mangifera indica* seed kernel extract in high-fat diet-induced obese mice. Pharmaceuticals (Basel) 13: 190. [Medline] [CrossRef]
37. Iglesias DE, Cremonini E, Hester SN, Wood SM, Bartlett M, Fraga CG, Oteiza PI. 2022. Cyanidin and delphinidin restore colon physiology in high fat diet-fed mice: involvement of TLR-4 and redox-regulated signaling. Free Radic Biol Med 188: 71–82. [Medline] [CrossRef]
38. Wernstedt Asterholm I, Tao C, Morley TS, Wang QA, Delgado-Lopez F, Wang ZV, Scherer PE. 2014. Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling. Cell Metab 20: 103–118. [Medline] [CrossRef]
39. Huang E, Kim S, Park H, Park S, Ji Y, Todorov SD, Lim SD, Holzapfel WH. 2021. Modulation of the gut microbiome and obesity biomarkers by *Lactobacillus plantarum* KC28 in a diet-induced obesity murine model. Probiotics Antimicrob Proteins 13: 677–697. [Medline] [CrossRef]
40. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R. 2008. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 57: 1470–1481. [Medline] [CrossRef]
41. Cremonini E, Wang Z, Bettaieb A, Adamo AM, Daveri E, Mills DA, Kalanetra KM, Haj FG, Karakas S, Oteiza PI. 2018. (-)-Epicatechin protects the intestinal barrier from high fat diet-induced permeabilization: implications for steatosis and insulin resistance. Redox Biol 14: 588–599. [Medline] [CrossRef]
42. Peng L, Li ZR, Green RS, Holzman IR, Lin J. 2009. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. J Nutr 139: 1619–1625. [Medline] [CrossRef]
43. Wang HB, Wang PY, Wang X, Wan YL, Liu YC. 2012. Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. Dig Dis Sci 57: 3126–3135. [Medline] [CrossRef]
44. Wang RX, Lee JS, Campbell EL, Colgan SP. 2020. Microbiota-derived butyrate dynamically regulates intestinal homeostasis through regulation of actin-associated protein synaptopodin. Proc Natl Acad Sci USA 117: 11648–11657. [Medline] [CrossRef]
45. Pryde SE, Duncan SH, Hold GL, Stewart CS, Flint HJ. 2002. The microbiology of butyrate formation in the human colon. FEMS Microbiol Lett 217: 133–139. [Medline] [CrossRef]
46. Bourriaud C, Robins RJ, Martin L, Kozlowski F, Tenailleau E, Cherbut C, Michel C. 2005. Lactate is mainly fermented to butyrate by human intestinal microfloras but inter-individual variation is evident. J Appl Microbiol 99: 201–212. [Medline] [CrossRef]
47. Duncan SH, Louis P, Flint HJ. 2004. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl Environ Microbiol 70: 5810–5817. [Medline] [CrossRef]
48. Delgado-Diaz DJ, Jesaveluk B, Hayward JA, Tyssen D, Alisoltani A, Potgieter M, Bell L, Ross E, Iranzadeh A, Allali I, Dabee S, Barnabas S, Gamieldien H, Blackburn JM, Mulder N, Smith SB, Edwards VL, Burgener AD, Bekker LG, Ravel J, Passmore JS, Masson L, Hearps AC, Tachedjian G. 2022. Lactic acid from vaginal microbiota enhances cervicovaginal epithelial barrier integrity by promoting tight junction protein expression. Microbiome 10: 141. [Medline] [CrossRef]
49. Huang Y, Zhang J, Dong R, Ji X, Jiang Y, Cen J, Bai Z, Hong K, Li H, Chen J, Zhou J, Qian F, Wang F, Qu Y, Zhou Y. 2021. Lactate as a metabolite from probiotic lactobacilli mitigates ethanol-induced gastric mucosal injury: an in vivo study. BMC Complement Med Ther 21: 26. [Medline] [CrossRef]
50. Ridlon JM, Kang DJ, Hylemon PB. 2006. Bile salt biotransformations by human intestinal bacteria. J Lipid Res 47: 241–259. [Medline] [CrossRef]
51. Ridlon JM, Harris SC, Bhowmik S, Kang DJ, Hylemon PB. 2016. Consequences of bile salt biotransformations by intestinal bacteria. Gut Microbes 7: 22–39. [Medline] [CrossRef]
52. Xie X, Zhang M, Sun L, Wang T, Zhu Z, Shu R, Wu F, Li Z. 2022. Crocin-I protects against high-fat diet-induced obesity *via* modulation of gut mmicrobiota and intestinal inflammation in mice. Front Pharmacol 13: 894089. [Medline] [CrossRef]
53. Tanaka A, Kanmura S, Morinaga Y, Kawabata K, Arima S, Sasaki F, Nasu Y, Tanoue S, Hashimoto S, Takeshita M, Takeda S, Ido A. 2020. Oral administration of *Lactobacillus plantarum* 06CC2 prevents experimental colitis in mice via an anti-inflammatory response. Mol Med Rep 21: 1181–1191. [Medline]
54. Madsen KL, Lewis SA, Tavernini MM, Hibbard J, Fedorak RN. 1997. Interleukin 10 prevents cytokine-induced disruption of T84 monolayer barrier integrity and limits chloride secretion. Gastroenterology 113: 151–159. [Medline] [CrossRef]

