

Berberine protects against diet-induced obesity through regulating metabolic endotoxemia and gut hormone levels

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Abstract. Systemic inflammation, which can be induced by metabolic endotoxemia, and corresponding high-fat diet-mediated metabolic disorders are associated with gut microbiota. In the present study reverse transcription-polymerase chain reaction, immunofluorescence, pyrosequencing, ELISA and Oil Red O staining were performed to assess whether berberine can protect against diet-induced obesity, through modulating the gut microbiota and consequently improving metabolic endotoxemia and gastrointestinal hormone levels. Alterations in the gut microbiota induced by berberine resulted in a significant reduction in bacterial lipopolysaccharide levels in portal plasma. Levels of inflammatory and oxidative stress markers, as well as the mRNA expression levels of macrophage infiltration markers in visceral adipose tissue, were also reduced by berberine. Inhibition of the inflammatory response was associated with a reduction in intestinal permeability and an increase in the expression of tight junction proteins. In addition, berberine was reported to restore aberrant levels of gut hormones in the portal plasma, such as glucagon-like peptide-1 and -2, peptide YY, glucose-dependent insulinotropic polypeptide and pancreatic polypeptide. The present findings indicated that berberine, through modulating gut microbiota, restored the gut barrier, reduced metabolic endotoxemia and systemic inflammation, and improved gut peptide levels in high-fat diet-fed rats. The present study suggests that berberine

may be an effective therapeutic strategy for the treatment of obesity and insulin resistance.

Introduction

Berberine, which is an alkaloid extracted from *Rhizoma coptidis*, has been traditionally used in Chinese medicine to treat gastrointestinal infections, due to its antimicrobial properties. Previous clinical research and animal studies have demonstrated that berberine can regulate glucose and lipid metabolism, and attenuate insulin resistance (1-3). Several mechanisms have been proposed to explain the actions of berberine in *in vitro* and *in vivo* models; these include: The activation of AMP-activated protein kinase to downregulate the expression of lipogenesis genes and upregulate the expression of energy expenditure genes (4); the inhibition of intestinal disaccharidases and α -glucosidase (5,6); the upregulation of the hepatic low-density lipoprotein receptor (7); the inhibition of intestinal cholesterol absorption (8); and increased intestinal glucagon-like peptide-1 (GLP-1) secretion (9,10). Since berberine has been reported to have poor intestinal absorption and very low absolute bioavailability, with values ranging between 0.36 and 0.68% in rats, it may be hypothesized that berberine exerts its effects in the intestinal tract prior to its absorption (11,12).

Accumulating evidence suggests that the gut microbiome serves an important role in obesity and related metabolic abnormalities. Taking into consideration the antibacterial activity of berberine, modulation of the gut microbiota has been suggested as another possible mechanism for its actions. Xie *et al* (2) reported that berberine significantly increased the intestinal expression of fasting-induced adipose factor (Fiaf), which acts as a lipoprotein lipase inhibitor, thereby inhibiting triglyceride deposition in adipocytes. Furthermore, it has been reported that *Lactobacillus paracasei* may upregulate Fiaf expression in colonic epithelial cells (13). These findings indicate that, through modulating the gut microbiota, berberine may increase the expression of Fiaf.

The leakage of bacterial-derived lipopolysaccharide (LPS) through the damaged intestinal mucosa into the circulation is a well-established mechanism of metabolic endotoxemia that can trigger systemic inflammation. Zhang *et al* (14) previously

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reported that berberine may prevent obesity and insulin resistance in high-fat diet (HFD)-fed rats by modulating the gut microbiota, thus contributing to the alleviation of inflammation via a reduction in serum LPS-binding protein and monocyte chemoattractant protein-1 (MCP-1). However, there is currently little information available on whether berberine can modulate endotoxemia and intestinal or systemic inflammation.

Previous studies have suggested that gut microbiota may contribute to the development of obesity and related disorders by modulating the synthesis of enteroendocrine peptides involved in glucose and energy homeostasis. A series of studies by Cani *et al* (15-18) reported that prebiotic use can interfere with plasma levels of intestinal peptides, causing an increase in GLP-1, GLP-2 and peptide YY (PYY), and a decrease in gastric inhibitory polypeptide (GIP) in rodent and human subjects. Short-chain fatty acids (SCFAs), produced during the bacterial fermentation of non-digestible carbohydrates, have been shown to promote GLP-1 and PYY secretion by stimulating the expression of G protein-coupled receptor 41 and 43 in enteroendocrine cells (L-cells) (19-22). Furthermore, prebiotics have been reported to promote GLP-2 production by increasing the number of intestinal L-cells and the mRNA expression of proglucagon (15). Taken together, these studies suggest that the fermentation of prebiotics by intestinal bacteria can interfere with gut peptide production. In addition, previous studies have revealed that berberine can increase the number of intestinal L-cells and thereby increase plasma GLP-1 levels in normal and diabetic rats (9,10,23). Furthermore, berberine has been demonstrated to promote ileal GLP-2 secretion and thus decrease LPS plasma levels in diabetic rats (24). Since GLP-2 is known to regulate the proliferation of intestinal epithelial cells and thus the integrity of the gut barrier, berberine may also promote intestinal integrity through modulating GLP-2 levels. Although previous studies have suggested that the effects of berberine on glucose metabolism and energy homeostasis are related to its modulatory effects on gut hormones, it remains to be elucidated whether other hormones may also be involved.

In order to investigate the effects of berberine administration on the gut and the gut microbiome, the present study employed a rat model of diet-induced obesity. Alterations in gut microbiota were assessed using 454 pyrosequencing, whereas intestinal hormone levels were assessed using Luminex technology. Intestinal permeability, the expression of tight junction proteins, endotoxemia, and systemic inflammation were also investigated.

Materials and methods

Materials. Berberine and fluorescein isothiocyanate (FITC)-dextran were purchased from Merck KGaA (Darmstadt, Germany). All diets were purchased from Research Diets, Inc. (New Brunswick, NJ, USA). Rat metabolic hormone kit, GLP-1 (cat. no. EGLP-35K) and GLP-2 (cat. no. EZGLP2-37K) ELISA kits were purchased from Merck KGaA. TRIzol[®] reagent and DAPI were purchased from Invitrogen (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Reverse transcription kit and SYBR-Green were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). Optimal cutting temperature (OCT) compound was

purchased from Sakura Finetek USA, Inc. (Torrance, CA, USA). Claudin1 (cat. no. ab203563), claudin2 (cat. no. ab53032) and GLP-1 antibodies (cat. no. ab22625) were purchased from Abcam (Cambridge, MA, USA). Goat anti-rabbit Cy3-conjugated secondary antibody (cat. no. 111-165-003) was purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA, USA). QIAamp DNA stool minikit was purchased from Qiagen, Inc. (Valencia, CA, USA). FastPfu polymerase was purchased from TransGen Biotech Co., Ltd. (Beijing, China). Axy-Prep DNA Gel Extraction kit was purchased from Axygen Biotechnology Co., Ltd. (Taizhou, China).

Animals. Thirty male Sprague-Dawley rats (age, 6 to 8 weeks; weight, ~260 g) were purchased from the SLAC Laboratory Animal Co., Ltd. (Shanghai, China), and were housed in a controlled environment (21 to 25°C; inverted 12-h daylight cycle; lights-off at 6:00 p.m.) in groups of 2 rats/cage and given free access to water and food. Following an acclimation period of 1 week, the rats were fed a control diet (Ctl group; n=10, 10% kcal from fat) or a HFD (HF group; n=20, 45% kcal from fat) for 14 weeks. Following 14 weeks, 10 rats from the HF group were maintained on the HFD however, they were given an oral supplement of berberine (150 mg/kg/day) for 6 weeks (HB group). For the duration of the study, the animals were weighed once a week, and their food intake was measured twice a week. All experimental procedures were validated by the Ethics Committee of Changhai Hospital, The Second Military Medical University (Shanghai, China).

Oral glucose tolerance test. The glucose tolerance tests were conducted following 6 weeks of berberine administration. Following a 12 h fast, the rats received an oral load of 50% glucose solution (2.0 g/kg). Blood glucose was sampled in the tail vein before and 15, 30, 60, 90 and 120 min following glucose administration with an ACCU-CHEK glucose meter (Roche Diagnostics, Basel, Switzerland).

In vivo intestinal permeability. Rats from all groups were fasted for 6 h and were subsequently administered FITC-dextran diluted in saline by gavage (500 mg/kg, 125 mg/ml). Following 1 and 4 h, 500 μ l of blood was sampled from the tail vein, placed in ice-cold heparinized tubes and centrifuged (12,000 \times g for 3 min at 4°C). The obtained plasma was then diluted with PBS (1:3 v/v) and the FITC-dextran concentration was determined using a fluorescence spectrophotometer (F7000; Hitachi, Ltd., Tokyo, Japan) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. A standard curve was obtained by diluting serial concentrations of FITC-dextran in non-treated plasma diluted with PBS (1:3 v/v).

Blood samples. At the end of the experiments, the rats were anesthetized by an intraperitoneal injection of 30 mg/kg pentobarbital following a 12-h fasting period. Blood samples were collected from the orbital plexus and the hepatic portal vein and centrifuged (2,000 \times g for 10 min at 4°C) to obtain plasma for further biochemical analyses. LPS concentration in portal plasma was determined using a kit utilizing *Tachypleus* amoebocyte lysate (Endosafe; Charles River Laboratories International, Inc., Wilmington, MA, USA) and

Table I. Primer sequences used for reverse transcription-quantitative polymerase chain reaction.

Gene	Forward primers (5'→3')	Reverse primers (5'→3')
TNF- α	TACTGAACTTCGGGGTGATTGGTCC	CAGCCTTGTCCCTTGAAGAGAACC
IL-1 β	GCTGTGGCAGCTACCTATGTCTTG	AGGTCGTCATCATCCCACGAG
PAI-1	AGTCTTTCCGACCAAGAGCA	CCAGTTTTGTCCCAAAGGAA
NADPHox	AAGTCATCCCCGCAACTGTTT	CCCGCTTCCTCATCTGCAATTC
STAMP-2	ATCCCATCAAAATTTGGCTT	CGCTGTGATTTGGAAGATTTAATAC
MCP-1	CAGATGCAGTTAATGCCCCAC	AGCCGACTCATTGGGATCAT
F4/80	CAGCTGTCTTCCCGACTTTC	TAATCAAGATTCCGGCCTTG
claudin-1	GCTGTCATCGGGGGCATAATA	CCTGGCATTGATAGGGGTCAT
claudin-2	GGACACTTATCAAGCGAG	CAGCAATGGGATTTAGACT
occludin	CCTCTGACCTTGTCCTGGATG	TCCCTGCTTTCCCTTCGTG
ZO-1	CTACCTTATTGAATGTC	AACTGAATGGTCTGATGCT
proglucagon	CCTCTATGCCAACACAGT	AGCCACCAATCCACACAG
β -actin	GGCTGGATTGTTTGTAAATGC	GGCGTTTGTCTTCGTTTATCT
GAPDH	GGCTCTCTGCTCCTCCCTGTTCTAG	CGTCCGATACGGCCAAATCCGT

TNF, tumor necrosis factor; IL, interleukin; PAI-1, plasminogen activator inhibitor-1; NADPHox, nicotinamide-adenine dinucleotide phosphate oxidase; STAMP-2, six transmembrane protein of prostate-2; MCP-1, monocyte chemotactic protein-1; F4/80, EGF-like module-containing mucin-like hormone receptor-like 1; ZO-1, zonula occludens-1.

estimated using the kinetic turbidimetric method. Intestinal hormone levels in portal plasma [total GIP, total pancreatic polypeptide (PP) and PYY] were determined in triplicate using a rat metabolic hormone kit (cat. no. RMHMAG-84K; Merck KGaA) and Luminex technology (Bio-Plex Multiplex system; Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's protocol. Total portal GLP-1 and GLP-2 levels were determined using ELISA kits. Plasma alanine triglycerides, cholesterol aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using an automatic biochemistry analyser (HITACHI 2000; Hitachi, Ltd.).

Tissue samples. The rats were anesthetized using chloral hydrate (400 mg/kg) and sacrificed by cervical dislocation. The visceral adipose tissue, and segments of the liver and proximal colon, were then removed. Tissues were immediately immersed in liquid nitrogen and stored at -80°C for further mRNA analysis. The remaining liver samples were used for hepatic lipid analysis and were stained with Oil Red O to detect fat droplets. The proximal colon samples were used for further immunofluorescence analysis.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from tissue samples using TRIzol[®] reagent according to the manufacturer's protocol. Total RNA (1 μg) was reverse transcribed into cDNA using the PrimeScript RT reagents kit (Takara Biotechnology Co., Ltd.). Briefly, the sample was incubated at 37°C for 15 min and then at 85°C for 5 sec. The mRNA levels of the different genes were examined using RT-qPCR. qPCR was conducted using the Rotor-Gene 3000 system and software (Qiagen, Inc., Valencia, CA, USA) using SYBR-Green. The thermocycling conditions were as follows: 95°C for 2 min, followed by 40 cycles of 95°C

for 10 sec, 55°C for 30 sec and 72°C for 30 sec. The primer sequences for the targeted genes are presented in Table I. The relative expression of each gene was normalized to the expression of the GAPDH gene and was calculated using the comparative Cq method ($\Delta\Delta\text{Cq}$) (25).

Oil Red O staining. Hepatic fat accumulation was evaluated by Oil Red O staining. Liver tissue was embedded in OCT compound and frozen in liquid nitrogen. The tissue was sliced into 8- μm cryostat sections and stained with 0.05% Oil Red O at room temperature for 30 min to detect lipid droplets. Photomicrographs were taken with a Nikon Eclipse E600 microscope (Nikon Corporation, Tokyo, Japan). The percentage of positively stained Oil Red O areas was quantified using the Image-Pro Plus software (version 6; Media Cybernetics, Inc., Rockville, MD, USA).

Immunofluorescence. Segments of the proximal colon were removed, washed with PBS and fixed immediately in 4% paraformaldehyde. The fixed tissue was dehydrated in ethanol, cleared in xylene, and embedded in paraffin. The paraffin sections (4- μm) were deparaffinized, rehydrated, treated with EDTA antigen retrieval buffers for 25 min at 4°C , and incubated with 5% bovine serum albumin (Sangon Biotech Co., Ltd., Shanghai, China) for 20 min to block non-specific binding. The slides were incubated with rabbit anti-claudin-1 (dilution, 1:300) or rabbit anti-claudin-2 primary antibodies (dilution, 1:300) overnight at 4°C in a moist chamber. The number of L-cells was determined by staining with rabbit anti-GLP-1 primary antibody (dilution, 1:300) overnight at 4°C . Subsequently, slides were washed 3 times with PBS and incubated with goat anti-rabbit Cy3-conjugated secondary antibody (dilution, 1:100) for 50 min at room temperature. The slides were washed a further 3 times with PBS, mounted

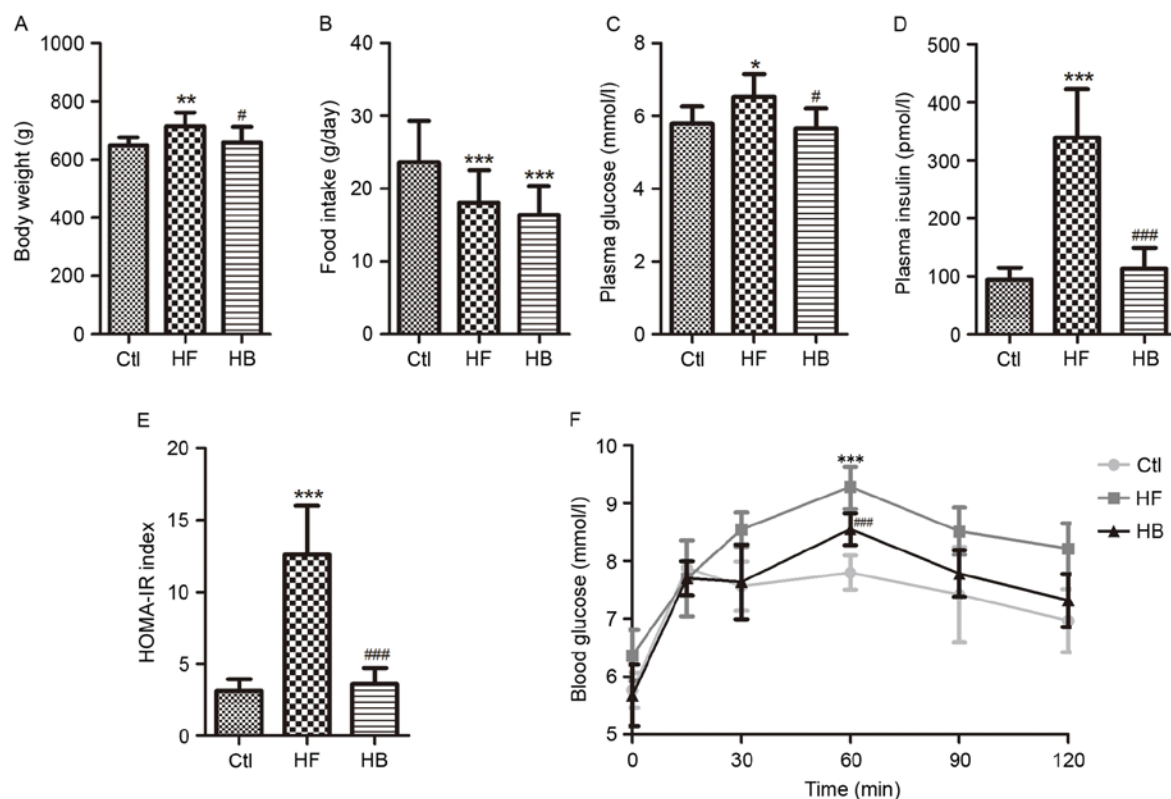


Figure 1. Berberine reduced weight gain and improved glucose homeostasis in HFD-fed rats. (A) Body weight. (B) Daily food intake per rat. (C) Fasting plasma glucose concentration. (D) Fasting plasma insulin concentration. (E) HOMA-IR index, calculated using the following equation: FBG (mmol/l) \times FINS (mU/l)/22.5. (F) Oral glucose tolerance test. Data are expressed as the mean \pm standard deviation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the Ctl group; # $P < 0.05$, ### $P < 0.001$ compared with the HF group. Ctl, normal diet; HF, HFD; HB, HFD supplemented with berberine for 6 weeks; HFD, high-fat diet; HOMA-IR, homeostasis model assessment of insulin resistance; FBG, fasting blood glucose; FINS, fasting blood insulin; OGTT, oral glucose tolerance test.

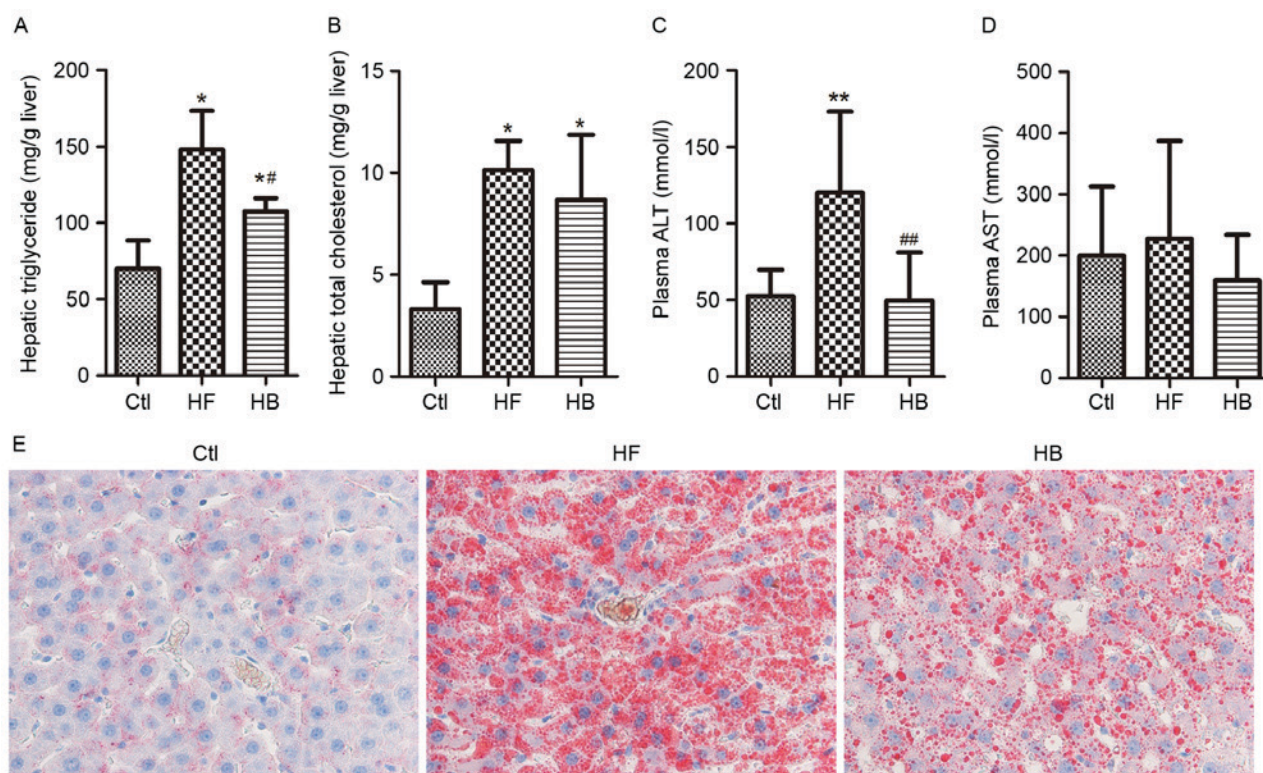


Figure 2. Berberine attenuated HFD-induced hepatic injury and hepatic steatosis. (A) Hepatic triglyceride content. (B) Hepatic total cholesterol content. (C) ALT plasma concentration. (D) AST plasma concentration. (E) Fresh-frozen liver sections were stained with Oil Red O (magnification, $\times 100$). Data are expressed as the mean \pm standard deviation. * $P < 0.05$, ** $P < 0.01$ compared with the Ctl group; # $P < 0.05$, ## $P < 0.01$ compared with the HF group. Ctl, normal diet; HF, HFD; HB, HFD supplemented with berberine for 6 weeks; HFD, high-fat diet; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

with ProLong Gold antifade reagent with DAPI, and analyzed under a Nikon Eclipse TE-2000-U fluorescent microscope (Nikon Corporation). A total of 5 fields from each intestinal segment were selected, and the mucosal area was manually delineated and measured by an image analyzer (Motic Image Plus 2.0ML; Motic Incorporation, Ltd., Causeway Bay, Hong Kong) for determining the number of L-cells. All stained samples were analyzed in a double-blind manner by 2 experienced investigators.

Pyrosequencing

DNA extraction from fecal samples. Cecal feces were collected from the caecum of each rat whilst under abdominal anaesthesia (30 mg/kg sodium pentobarbital) and were stored at -80°C prior to analysis. The total bacterial genomic DNA was extracted from the frozen feces (200 mg) using the QIAamp DNA stool minikit according to the manufacturer's protocol.

PCR amplification of 16S rRNA and pyrosequencing. The extracted DNA served as a template to amplify the V1-3 region of the 16S rRNA gene. The primers used were as follows: Forward primer 5'-NNNNNNNNAGAGTTTGATCCTGGC TCAG-3' and reverse primer 5'-NNNNNNNNTTACCGC GGCTGCTGGCAC-3'. NNNNNNNN indicates the 8-base bar code sequence used to tag each PCR product, and the underlined sequence indicates the broad-range primers used to amplify the V1-3 region of the 16S rRNA gene. The PCR amplification mixture (20 μl) contained 10 ng template DNA, 4 μl 5X PCR FastPfu buffer, 0.2 units FastPfu polymerase, 2.5 mM dNTP mixture and 0.4 μM of each primer. The PCR reactions were performed using a GeneAmp PCR system 9700 cyclor (Applied Biosystems; Thermo Fisher Scientific, Inc.). The PCR conditions were as follows: Denaturation at 95°C for 2 min, followed by 25 cycles at 95°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec, and a final extension at 72°C for 5 min. The PCR products were separated by electrophoresis and subsequently cut from the 2% agarose gel. The products were purified using the Axy-Prep DNA Gel Extraction kit. The purified DNA was quantified using the QuantiFluor system (Promega Corporation, Madison, WI, USA). A total of 4 μg purified DNA was added to a master pool, and the DNA pool was sent to Major Biosystem Co., Ltd., (Taipei, Taiwan) for pyrosequencing using the GS FLX system (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's protocol. The company analysed the data using Weighted UniFrac principal coordinates analysis (PCoA), principal component analysis (PCA), redundancy analysis (RDA), Monte Carlo permutation and Mothur tests.

Statistical analysis. Experiments were repeated at least 3 times and data are expressed as the mean \pm standard deviation. $P < 0.05$ was considered to indicate a statistically significant difference. The statistical significance of the difference between groups was assessed by one-way analysis of variance, followed by a post hoc Bonferroni's multiple comparison tests; or by Kruskal-Wallis test for non-parametric data, followed by a Dunn's multiple comparison test. Correlations between parameters were assessed by the Spearman's correlation coefficient. The analysis was performed using SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA) and figures were

created using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Berberine prevents obesity and improves glucose homeostasis in HFD-fed rats. Rats maintained on a HFD for 14 weeks exhibited a significant increase in body weight compared with the control rats (636.26 ± 44.83 vs. 591.34 ± 30.65 g; $P < 0.01$; Fig. 1A). Treatment with berberine for 6 weeks significantly reversed the body weight increase of HFD-fed rats compared with untreated HFD-fed rats (658.58 ± 54.04 vs. 715.59 ± 46.70 g; $P < 0.05$; Fig. 1A). As a result, rats in the HB treatment group had a body weight similar to the control group (Fig. 1A). During the course of the study, food intake was monitored twice a week. Average daily food intake appeared to be smaller in the HB treatment group; however, no significant difference was revealed when compared with the HF group (Fig. 1B). These results suggested that berberine may prevent obesity without interfering with food intake.

Fasting blood glucose and fasting blood insulin appeared to be significantly increased in rats of the HF group compared with the control group (Fig. 1C and D). The HFD also caused impaired glucose tolerance and insulin resistance, which was apparent by the significantly increased homeostatic model assessment of insulin resistance index and the significantly increased area under the curve following glucose challenge (Fig. 1E and F). Berberine treatment significantly improved fasting blood insulin and insulin resistance, however, not fasting blood glucose (Fig. 1C-F).

Berberine alleviates HFD-induced hepatic steatosis and injury. Hepatic steatosis was evaluated by measuring hepatic triglyceride contents via Oil Red O staining, whereas hepatic injury was evaluated by measuring circulating liver enzyme levels. Rats maintained on a HFD developed hepatic steatosis and injury, as reflected by the significantly increased hepatic triglyceride contents and ALT levels (Fig. 2A-D). Furthermore, Oil Red O staining demonstrated that large lipid droplets accumulated in the liver of HFD-fed rats (Fig. 2E). Berberine supplementation significantly decreased plasma ALT levels however, it did not affect aspartate aminotransferase levels when compared with untreated HFD-fed rats (Fig. 2C and D). A marked decrease in the amount of Oil Red O-stained lipid droplets in the berberine-treated group was also observed (Fig. 2E). These results suggested that HFD-induced hepatic steatosis and injury may be significantly alleviated by berberine.

Berberine reduces endotoxemia and visceral adipose tissue inflammation in HFD-fed rats. LPS levels in portal plasma were significantly higher in HFD-fed rats compared with control rats (Fig. 3A). Following treatment with berberine for 6 weeks, LPS plasma levels in HFD-fed rats were significantly reduced when compared with the untreated HFD-fed rats; however, LPS levels in berberine-treated rats remained higher than in control rats. With regards to inflammation and oxidative stress in visceral adipose tissue, and their role in obesity and insulin resistance, the following seven genes were investigated: Tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β),

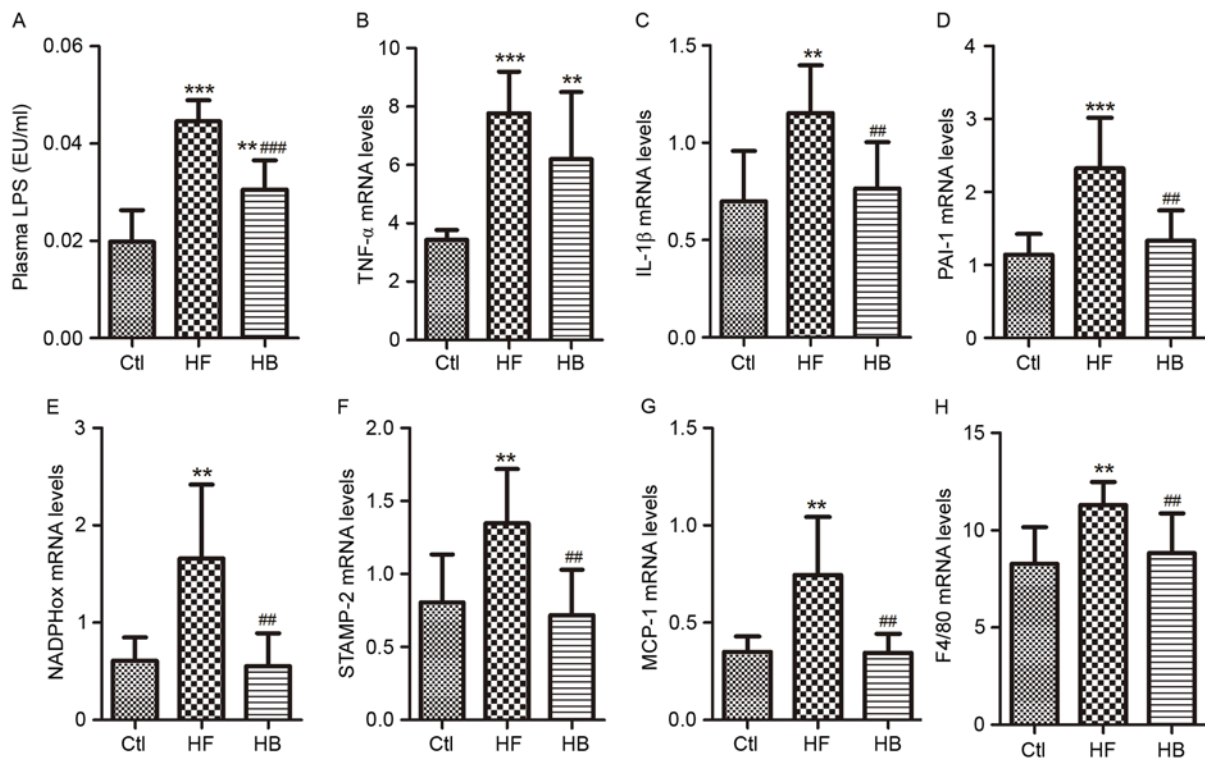


Figure 3. Berberine reduced endotoxemia and visceral adipose tissue inflammation in HFD-fed rats. (A) Portal plasma LPS concentrations. (B) TNF- α mRNA expression levels. (C) IL-1 β mRNA expression levels. (D) PAI-1 mRNA expression levels. (E) NADPHox mRNA expression levels. (F) STAMP-2 mRNA expression levels. (G) MCP-1 mRNA expression levels. (H) F4/80 mRNA expression levels. Data are expressed as the mean \pm standard deviation. ** P <0.01, *** P <0.001 compared with the Ctl group; ## P <0.01, ### P <0.001 compared with the HF group. Ctl, normal diet; HF, HFD; HB, HFD supplemented with berberine for 6 weeks; HFD, high-fat diet; LPS, lipopolysaccharide; EU, endotoxin unit; TNF, tumor necrosis factor; IL, interleukin; PAI-1, plasminogen activator inhibitor-1; NADPHox, nicotinamide-adenine dinucleotide phosphate oxidase; STAMP-2, six transmembrane protein of prostate-2; MCP-1, monocyte chemoattractant protein-1; F4/80, EGF-like module-containing mucin-like hormone receptor-like 1.

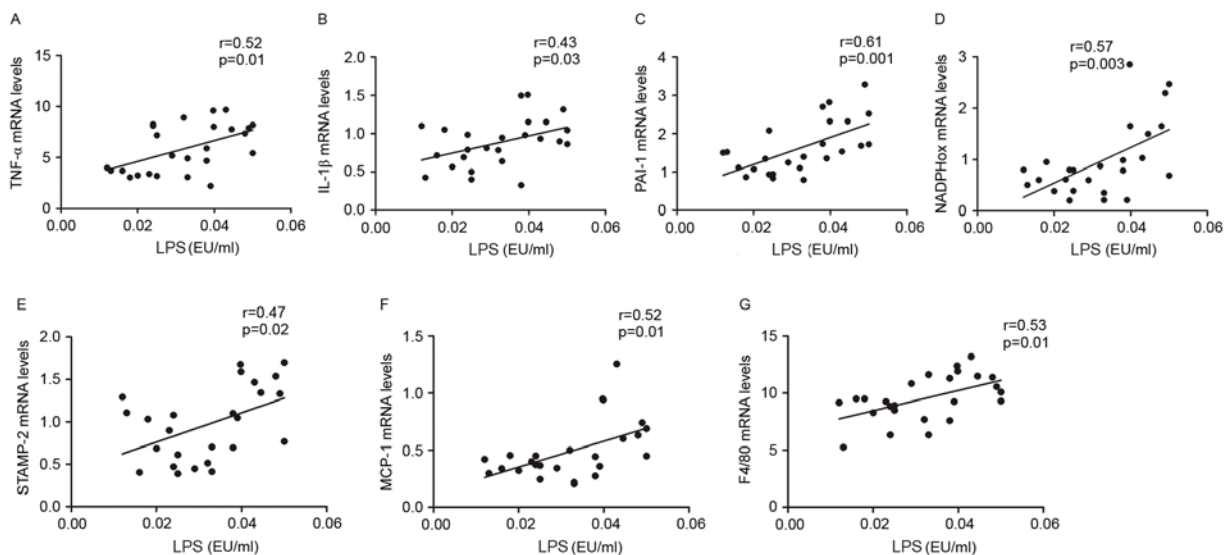


Figure 4. Metabolic endotoxemia was positively correlated with inflammation, oxidative stress and macrophage infiltration markers. Correlation between: Portal plasma LPS level and (A) TNF- α , (B) IL-1 β , (C) PAI-1, (D) NADPHox, (E) STAMP-2, (F) MCP-1 and (G) F4/80 mRNA levels in visceral adipose tissue of Ctl, HF and HB rats. The inset corresponds to Pearson's correlation and corresponding P-value. Ctl, normal diet; HF, HFD; HB, HFD supplemented with berberine for 6 weeks; HFD, high-fat diet; LPS, lipopolysaccharide; TNF, tumor necrosis factor; IL, interleukin; PAI-1, plasminogen activator inhibitor-1; NADPHox, nicotinamide-adenine dinucleotide phosphate oxidase; STAMP-2, six transmembrane protein of prostate-2; MCP-1, monocyte chemoattractant protein-1; F4/80, EGF-like module-containing mucin-like hormone receptor-like 1; EU, endotoxin unit.

plasminogen activator inhibitor-1 (PAI-1), six transmembrane protein of prostate-2 (STAMP-2), nicotinamide-adenine dinucleotide phosphate oxidase (NADPHox), MCP-1 and EGF-like

module-containing mucin-like hormone receptor-like 1 (F4/80). In visceral adipose tissue samples, the mRNA expression levels of these genes were significantly increased

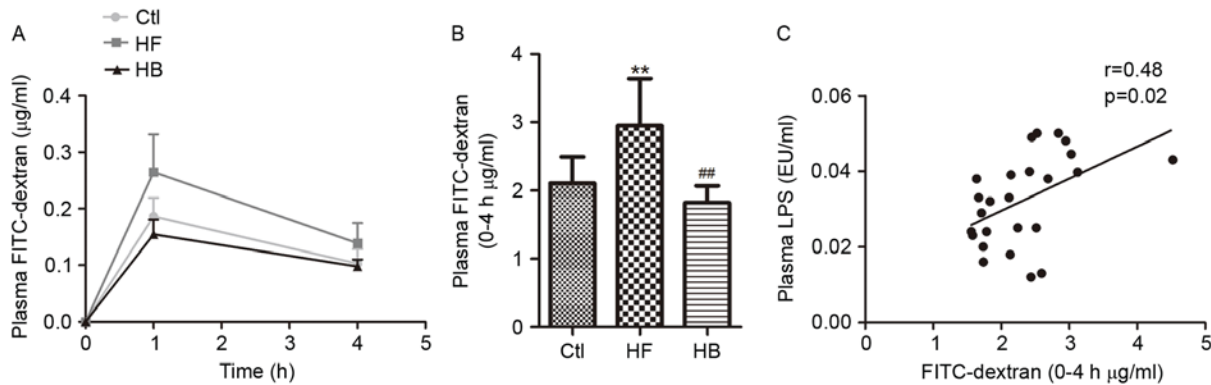


Figure 5. Berberine reduced intestinal permeability in HFD-fed rats. (A) Plasma FITC-dextran concentration. (B) Total plasma FITC-dextran concentration. (C) Correlation between portal plasma LPS levels and plasma FITC-dextran concentration. The inset corresponds to Pearson's correlation and the corresponding P-value. Data are expressed as the mean \pm standard deviation. ** $P < 0.01$ compared with the Ctl group; ## $P < 0.01$ compared with the HF group. Ctl, normal diet; HF, HFD; HB, HFD supplemented with berberine for 6 weeks; HFD, high-fat diet; FITC, fluorescein isothiocyanate; LPS, lipopolysaccharide; EU, endotoxin unit.

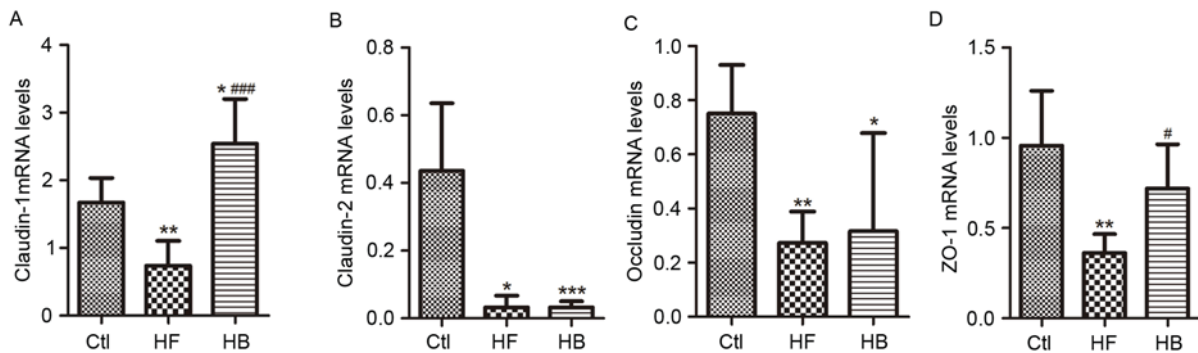


Figure 6. Berberine restored the expression of tight junction proteins in HFD-fed rats. mRNA expression levels of colonic epithelial tight junction proteins (A) claudin-1, (B) claudin-2, (C) occludin and (D) ZO-1. Data are expressed as the mean \pm standard deviation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the Ctl group; # $P < 0.05$, ### $P < 0.001$ compared with the HF group. Ctl, normal diet; HF, HFD; HB, HFD supplemented with berberine for 6 weeks; HFD, high-fat diet; ZO-1, zonula occludens-1.

in HFD-fed rats compared with in control rats (Fig. 3B-H). Treatment with berberine significantly reduced IL-1 β , PAI-1, STAMP-2, NADPHox, MCP-1 and F4/80 mRNA expression levels compared with in untreated HFD-fed rats (Fig. 3C-H). Berberine appeared to have no effect on TNF- α mRNA levels compared with in untreated HFD-fed rats (Fig. 3B).

To explore whether endotoxemia affected inflammatory processes in visceral adipose tissue, the correlation between LPS plasma levels and TNF- α , IL-1 β , PAI-1, STAMP-2, NADPHox, MCP-1 and F4/80 mRNA expression levels was investigated. The present results indicated that LPS portal plasma levels were positively correlated with the mRNA expression levels of TNF α , IL-1 β , PAI-1, NADPHox, STAMP2, MCP-1 and F4/80 in visceral adipose tissue (Fig. 4A-G). These multiple correlations suggested that gut microbiota and endotoxemia may synergistically contribute to inflammation, oxidative stress and macrophage infiltration in HFD-fed rats.

Berberine reduces intestinal permeability and ameliorates the expression and distribution of tight junction proteins in HFD-fed rats. To investigate whether endotoxemia could exert an effect on intestinal permeability, the plasma concentration of FITC-dextran was examined. In accordance with the changes in plasma LPS levels, a marked increase in plasma

FITC-dextran area under the curve was observed in HFD-fed rats compared with in the control rats (Fig. 5A). Treatment with berberine significantly reduced plasma FITC-dextran concentration in the HFD-fed rats compared with in untreated rats (Fig. 5A and B). Furthermore, portal plasma LPS levels appeared to be positively correlated with plasma FITC-dextran concentration (Fig. 5C). These findings suggested that berberine may reduce HFD-induced endotoxemia, through interfering with the control of intestinal permeability.

Tight junction proteins control paracellular permeability. In the present study, the effect of berberine on the expression and distribution of candidate tight junction proteins, including claudin-1, claudin-2, zonula occludens-1 (ZO-1) and occludin, was investigated using RT-qPCR and immunofluorescence. Claudin-1, claudin-2, ZO-1 and occludin mRNA expression levels in the proximal colon segments from HFD-fed rats were significantly decreased, as compared with rats in the control group. Treatment with berberine appeared to restore claudin-1 and ZO-1 mRNA expression levels, however, it had no significant effect on claudin-2 and occludin mRNA expression levels (Fig. 6A-D).

As shown in Fig. 7, immunofluorescence revealed that the tight junction proteins claudin-1 and claudin-2 are normally distributed along the epithelial sheet from the crypt to the

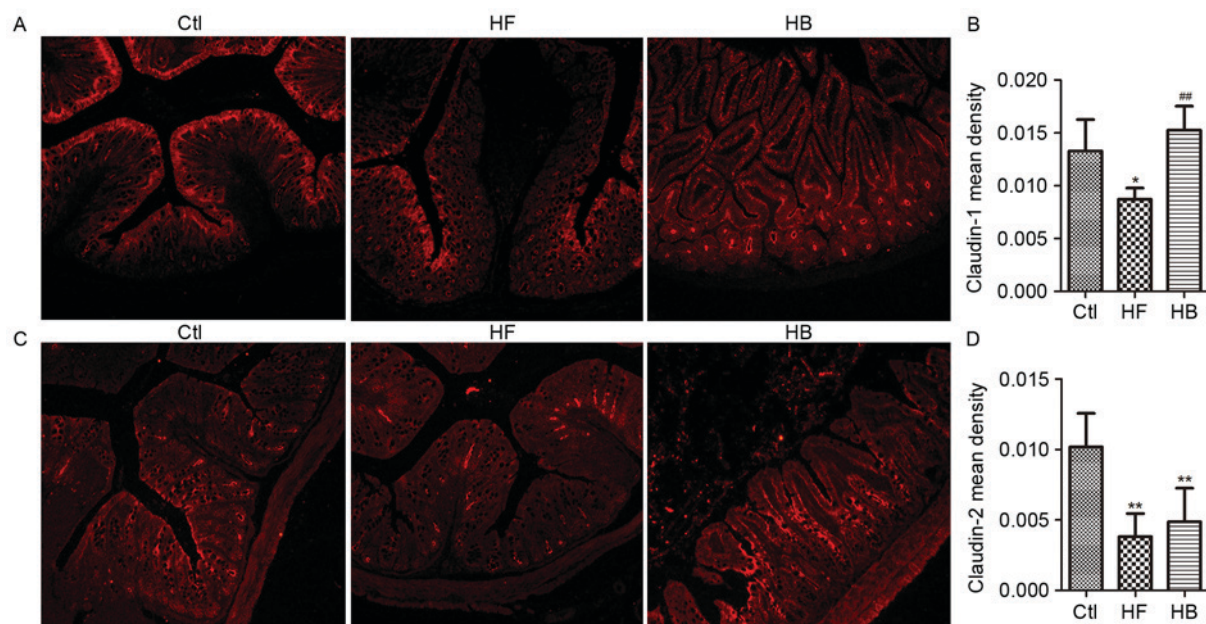


Figure 7. Berberine restored the expression and distribution of claudin-1 and claudin-2 in HFD-fed rats. (A) Immunofluorescent staining for claudin-1 (magnification, x100). (B) Immunohistochemistry score for claudin-1. (C) Immunofluorescent staining for claudin-2 (magnification, x100). (D) Immunohistochemistry score for claudin-2. Data are expressed as the mean \pm standard deviation. * $P < 0.05$, ** $P < 0.01$ compared with the Ctl group; *** $P < 0.001$ compared with the HF group. Ctl, normal diet; HF, HFD; HB, HFD supplemented with berberine for 6 weeks; HFD, high-fat diet.

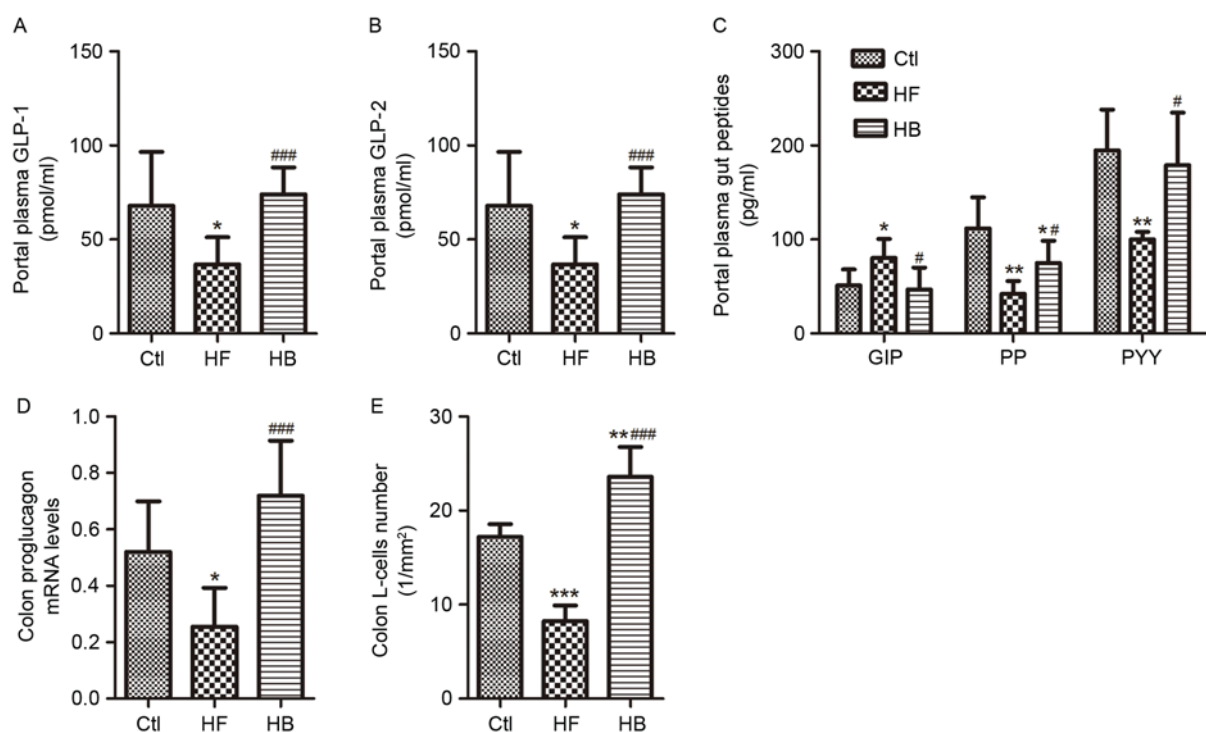


Figure 8. Berberine modulated intestinal hormone levels in portal plasma of HFD-fed rats. (A) Portal plasma GLP-1 levels. (B) Portal plasma GLP-2 levels. (C) Portal plasma GIP, PP and PYY levels. (D) Colonic proglucagon mRNA expression levels. (E) Colonic L-cell numbers. Data are expressed as the mean \pm standard deviation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the Ctl group; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ compared with the HF group. Ctl, normal diet; HF, HFD; HB, HFD supplemented with berberine for 6 weeks; HFD, high-fat diet; GLP, glucagon-like peptide; GIP, gastric inhibitory polypeptide; PP, pancreatic polypeptide; PYY, peptide YY.

villous, and the staining for both proteins appeared continuous and dense. Conversely, in HFD-fed rats, the staining revealed a translocation of claudin-1 and claudin-2 from the tight junction to the luminal side of the crypt. Tissue from berberine-treated rats exhibited strong claudin-1 and claudin-2 staining in the

villous surface, similar to the control group (Fig. 7A and C). These observations suggested that berberine treatment may attenuate the HFD-induced redistribution of claudin-1 and claudin-2. Furthermore, in accordance with the mRNA analysis results, the immunohistochemical staining scores (quantified

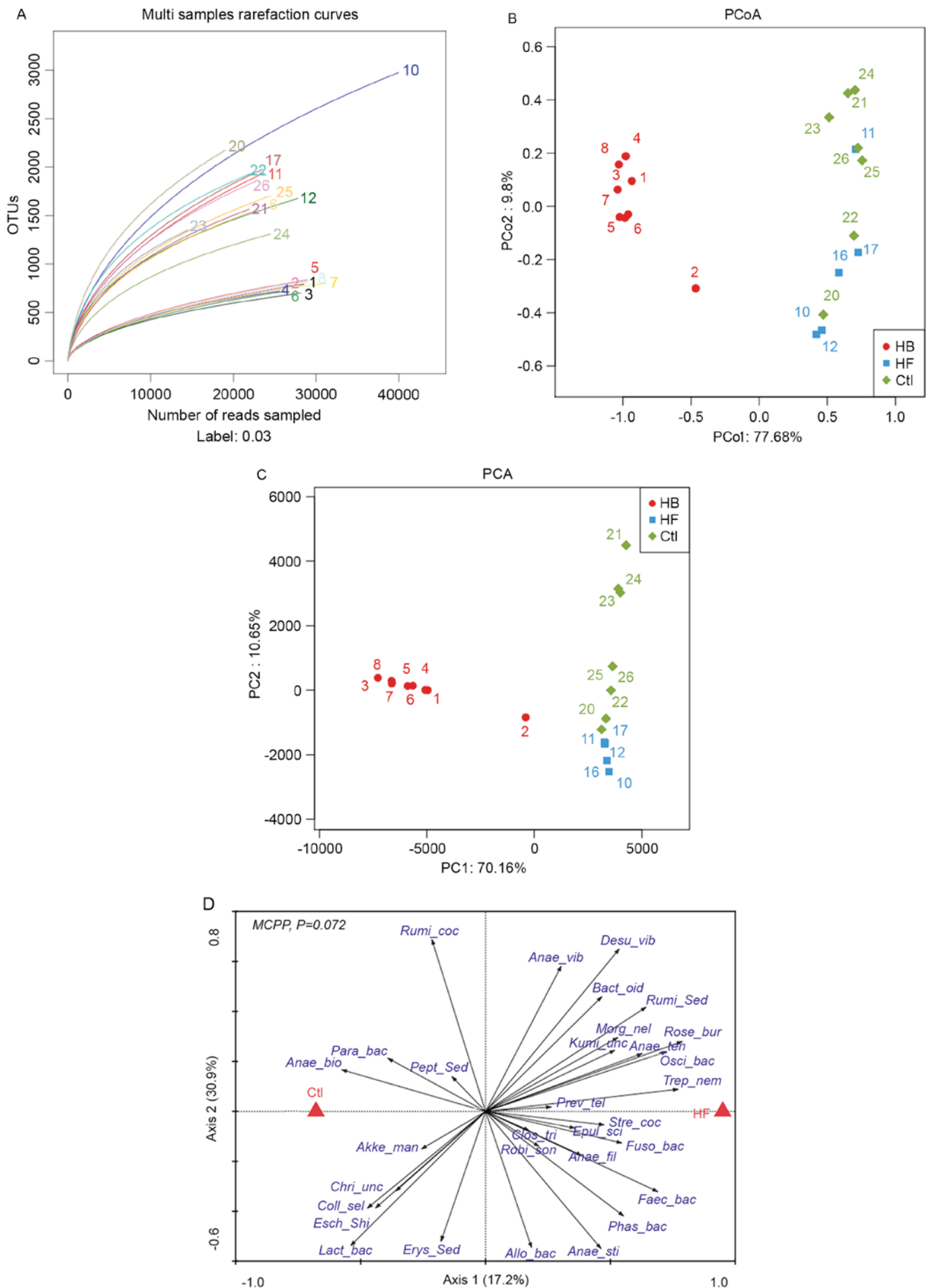


Figure 9. Berberine altered the gut microbiota composition in HFD-fed rats. (A) Rarefaction curves. (B) Weighted UniFrac PCoA. Each point represents the microbiota of each rat in the Ctl, HF and HB groups. (C) PCA. P-values were calculated by MCPP. (D) RDA between the Ctl and HF groups.

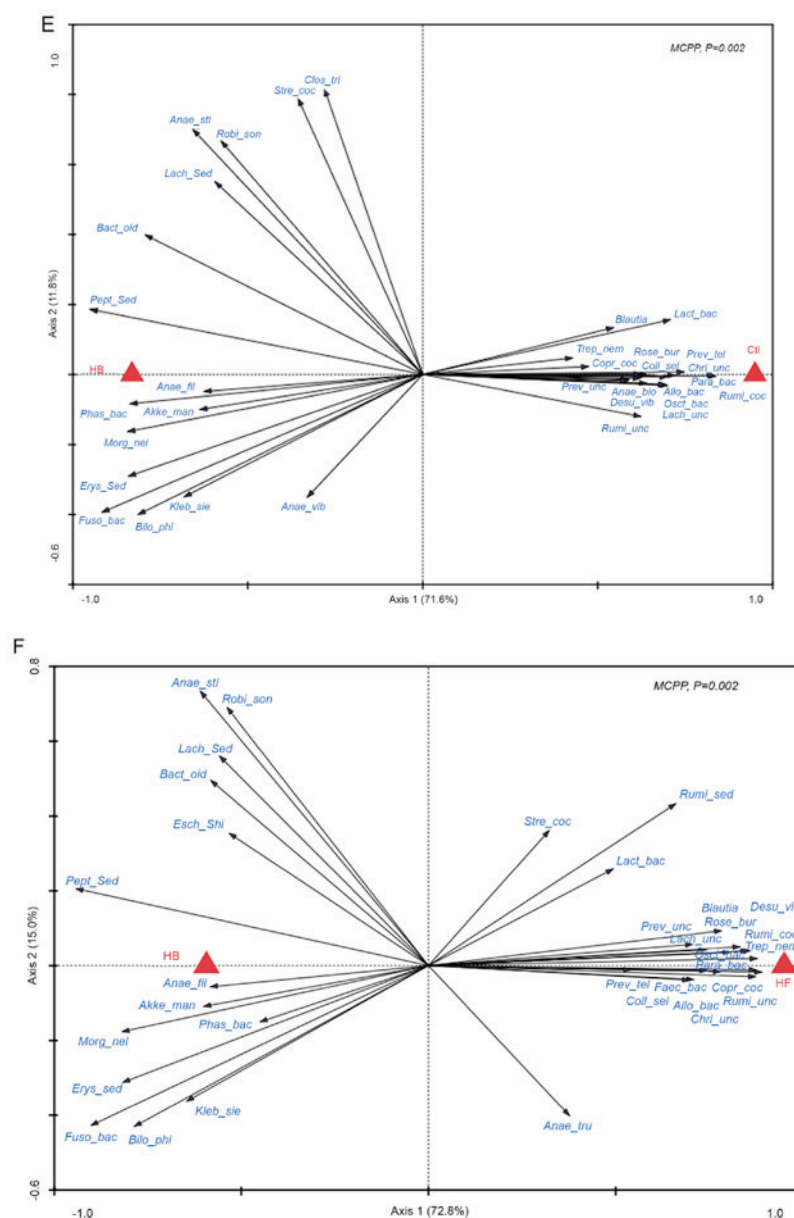


Figure 9. Continued. Berberine altered the gut microbiota composition in HFD-fed rats. (E) RDA between the Ctl and HB groups. (F) RDA between the HB and HF groups. P-values were calculated by MCPP. Ctl, normal diet; HF, HFD; HB, HFD supplemented with berberine for 6 weeks; HFD, high-fat diet; PCoA, principal coordinates analysis; PCA, principal components analysis; RDA, redundancy analysis; MCPP, Monte Carlo permutation tests.

by v.6 Image-Pro Plus software) for claudin-1 protein appeared significantly higher in berberine-treated rats compared with in untreated HFD-fed rats (Fig. 7B and D).

Berberine modulates intestinal hormone levels in portal plasma. Levels of the intestinal hormones GLP-1, GLP-2, PP and PYY appeared to be significantly reduced, whereas GIP levels were significantly increased in portal plasma samples of HFD-fed rats. Treatment with berberine restored the concentrations of GLP-1, GLP-2, PYY and GIP to those of the control rats (Fig. 8A-C). Furthermore, berberine almost doubled the portal plasma GLP-1 and GLP-2 levels as compared with HFD-fed rats. Berberine supplementation increased PP plasma levels significantly (Fig. 8C). In addition, berberine-treated rats exhibited a 3-fold increase in proglucagon mRNA expression levels and in the number of GLP-1-positive L-cells in

the proximal colon compared with HFD-fed untreated rats (Fig. 8D and E).

Berberine alters the composition of the gut microbiome. In order to investigate the effects of a HFD and berberine intake on the composition of the gut microbiome, 454 pyrosequencing of the bacterial 16S rRNA gene V1-V3 region was performed. A total of 602,852 usable reads (119,071 unique sequences) obtained from 20 samples were delineated into 10,385 operational taxonomic units (OTUs) at the 97% similarity level. Rarefaction curves indicated that most of the diversity of each microbial group was successfully sampled (Fig. 9A). Berberine supplementation appeared to cause a significant reduction in the richness of the gut microbiota, as compared with HFD-fed untreated rats, which was confirmed by Ace and Chao estimators (Table II). Berberine treatment

Table II. Community richness and diversity estimator.

Sample ID ^a	Reads ^b	OTU numbers	Ace	Chao	Coverage	Shannon	Simpson
HB group							
1	28,506	794	1,932	1,493	0.986038	3.45	0.0915
2	26,026	759	2,039	1,394	0.985361	4.07	0.0376
3	27,962	697	1,462	1,234	0.988556	3.12	0.1485
4	25,582	721	1,385	1,145	0.987726	3.32	0.1273
5	28,941	836	1,867	1,406	0.986179	3.5	0.1033
6	26,300	719	1,560	1,153	0.987262	3.36	0.1037
7	31,163	805	1,636	1,310	0.988223	3.4	0.1044
8	28,936	819	1,795	1,354	0.986557	3.28	0.1215
HF group							
10	39,945	2976	6,450	5,108	0.965578	5.71	0.0151
11	23,455	1931	4,621	3,464	0.959497	5.45	0.0173
12	27,799	1676	3,183	2,641	0.975143	5.47	0.0153
16	22,767	1558	3,283	2,577	0.969254	5.04	0.0389
17	23,426	1976	4,493	3,385	0.959959	5.59	0.012
Ctl group							
20	19,005	2174	4,293	3,686	0.949277	6.2	0.0056
21	21,938	1567	3,157	2,568	0.968183	4.54	0.0821
22	22,005	1930	3,442	2,941	0.964281	5.7	0.0145
23	14,470	1349	2,441	2,039	0.960539	4.47	0.114
24	24,471	1310	2,556	2,186	0.976585	4.61	0.0424
25	24,497	1704	3,447	2,924	0.968813	5.16	0.0217
26	22,357	1842	3,873	3,162	0.961936	5.28	0.0218

^aSample names. ^bTrimmed sequence numbers assigned to OTUs. OTU, operational taxonomic unit; HB, high fat diet supplemented with berberine for 6 weeks; HF, high fat diet; Ctl, normal diet.

also appeared to significantly reduce the diversity of the gut microbiota, which was confirmed by the Shannon and Simpson indices (Table II).

A total of 10,119 OTUs (contributing to 98.8% of all sequencing reads) were assigned to 19 phyla by Mothur analysis. The most abundant phyla included *Firmicutes* (7,432 OTUs, 70.3% of all reads), *Bacteroidetes* (1,044 OTUs, 10.4% of all reads), *Fusobacteria* (457 OTUs, 8.2% of all reads), *Proteobacteria* (509 OTUs, 6.1% of all reads) and *Actinobacteria* (348 OTUs, 3.0% of all reads). As revealed by taxon-based analysis, there was a significant decrease in the abundance of the *Actinobacteria* phylum in the HFD-fed group compared with in the control group, whereas no significant differences were observed in the *Firmicutes*, *Bacteroidetes*, *Fusobacteria* and *Proteobacteria* phyla (Table III). Berberine markedly altered the gut microbiota composition at the phylum level, significantly increasing the abundance of *Fusobacteria* and *Proteobacteria*, and decreasing the abundance of *Firmicutes* and *Actinobacteria* (Table III). Berberine had no effect on the abundance of the *Bacteroidetes* phylum. In addition, berberine appeared to significantly affect the abundance of 59 genera (Table III). Among these, 12 genera displayed a 10-fold increase, and 37 genera displayed a 10-fold decrease in average frequency, compared with the HFD group rats. Furthermore, 37 genera were identified exclusively in

berberine-treated rats, whereas 18 genera were identified exclusively in HFD-fed untreated animals.

Weighted UniFrac PCoA was performed to provide an overview of the gut microbiota composition. The gut microbiota composition changed significantly in response to HFD and berberine administration (Fig. 9B). PCo 1 (accounting for 77.68% of total variance) mainly reflected the effects of berberine on gut microbiota composition, as PCo 1 separated the HB group from the HF and Ctl groups. PCo 2 (accounting for 9.8% of total variance) mainly reflected the effect of different diets, as PCo 2 separated the Ctl group from the HB and HF groups. These results suggested that berberine can shift the composition of the gut microbiome of HFD-fed rats towards that of control rats. Similar results were obtained from the PCA (Fig. 9C).

Specific genera of gut bacteria responded to treatment with berberine. RDA was used to identify specific bacterial genera whose abundance was affected by HFD or berberine supplementation. Results indicated that a HFD caused a slight change in the composition of gut microbiota (Fig. 9D), whereas berberine treatment led to a significant change in the gut microbiota composition (Fig. 9E), which was proven by the Monte Carlo permutation tests (MCP; $P=0.002$). Following treatment with berberine, the composition of the

Table III. Differentially abundant features analysis at the phylum or genus level.

Phylum/Genus	HB (%) mean ± SD	HF (%) mean ± SD	Ctl (%) mean ± SD	P-value		
				HB vs. HF	HF vs. Ctl	HB vs. Ctl
Phylum						
<i>Acidobacteria</i>	0.00135±0.00066	0.00293±0.0008	0.00199±0.00095	0.43870	0.71705	0.68879
<i>Actinobacteria</i>	0.02004±0.00564	1.94705±0.72971	7.24482±2.45339	0.01408	0.04507	0.00631
<i>Bacteroidetes</i>	11.37515±2.19301	14.64713±3.97861	7.62278±1.86003	0.51500	0.11850	0.18400
<i>TM7</i>	0.00182±0.0012	0.18116±0.09689	1.89118±0.6578	0.06685	0.01307	0.00685
<i>Chloroflexi</i>	0.00043±0.00043	0±0	0.00058±0.00058	1.00000	1.00000	1.00000
<i>Cyanobacteria</i>	0.00095±0.00095	0.08708±0.06498	0.06448±0.02119	0.20177	0.74114	0.00562
<i>Deferribacteres</i>	0±0	0.00314±0.00194	0.00756±0.00683	0.02102	0.57029	0.25377
<i>Deinococcus-Thermus</i>	0.00137±0.00095	0.00085±0.00085	0.00065±0.00065	1.00000	1.00000	1.00000
<i>Elusimicrobia</i>	0±0	0.00207±0.00089	0.00232±0.00114	0.05521	1.00000	0.06384
<i>Firmicutes</i>	34.07855±3.97821	79.44588±4.13566	79.69249±2.76575	0.00000	0.89414	0.00000
<i>Fusobacteria</i>	40.28194±4.39043	0.12066±0.05127	0.0163±0.00883	0.00000	0.05379	0.00000
<i>Gemmatimonadetes</i>	0.00095±0.00095	0±0	0.00058±0.00058	0.52842	1.00000	1.00000
<i>Lentisphaerae</i>	0±0	0.00968±0.00386	0.00389±0.00251	0.01669	0.22271	0.00408
<i>Nitrospirae</i>	0±0	0.00072±0.00072	0±0	0.38079	0.48016	1.00000
<i>Planctomycetes</i>	0.00044±0.00044	0.00157±0.00097	0±0	0.56199	0.23056	1.00000
<i>Proteobacteria</i>	11.05874±1.14416	2.45033±0.51587	2.29854±0.57357	0.00000	0.81821	0.00000
<i>Spirochaetes</i>	0.0013±0.0013	0.89612±0.16617	0.20734±0.11901	0.00015	0.00286	0.07762
<i>Tenericutes</i>	0.013±0.01151	0.12251±0.08587	0.88127±0.36557	0.22892	0.05143	0.02031
<i>Verrucomicrobia</i>	3.14836±1.01832	0.00072±0.00072	0.00646±0.00424	0.00469	0.19707	0.00492
Genus						
<i>Acetanaerobacterium</i>	0.00595±0.00473	0±0	0±0	0.22569	1.00000	0.22139
<i>Acetobacteraceae_uncultured</i>	0±0	0±0	0.00065±0.00065	1.00000	1.00000	0.39968
<i>Acholeplasma</i>	0±0	0±0	0.00117±0.00117	1.00000	0.50079	0.15974
<i>Acidobacteriaceae_uncultured</i>	0±0	0±0	0.00075±0.00075	1.00000	1.00000	0.39968
<i>Acidothermus</i>	0±0	0.00072±0.00072	0±0	0.38079	0.48016	1.00000
<i>Acidovorax</i>	0.00048±0.00048	0±0	0.00058±0.00058	1.00000	1.00000	1.00000
<i>Acinetobacter</i>	0.00179±0.00133	0±0	0±0	0.30479	1.00000	0.15540
<i>Actinomyces</i>	0.00315±0.00127	0.01949±0.01252	0.00564±0.00313	0.21147	0.30068	0.55334
<i>Adlercreutzia</i>	0±0	0.00562±0.00501	0.03181±0.00825	0.29136	0.01312	0.00060
<i>Aeribacillus</i>	0.00086±0.00086	0.00088±0.00088	0±0	1.00000	0.48016	0.52013
<i>Aerococcaceae_uncultured</i>	0.00044±0.00044	0±0	0±0	1.00000	1.00000	1.00000
<i>Aerococcus</i>	0±0	0.00088±0.00088	0±0	0.38079	0.48016	1.00000
<i>Agrococcus</i>	0.00043±0.00043	0±0	0±0	1.00000	1.00000	1.00000
<i>Akkermansia</i>	3.14836±1.01832	0±0	0.00447±0.00447	0.00472	0.34499	0.00353
<i>Alcaligenes</i>	0±0	0.00288±0.00288	0±0	0.02102	0.05316	1.00000
<i>Alistipes</i>	0±0	0.00423±0.00266	0.02031±0.00915	0.12302	0.10822	0.03099
<i>Allobaculum</i>	0.00311±0.00079	10.84206±3.94134	8.82073±2.9406	0.01252	0.74820	0.00424
<i>Anaerobiospirillum</i>	0.00049±0.00049	0.00088±0.00088	0.46779±0.17407	1.00000	0.01388	0.00936
<i>Anaerofilum</i>	2.30376±0.73263	0.05499±0.02737	0.0219±0.01234	0.00482	0.28611	0.00332
<i>Anaerofustis</i>	0±0	0.01313±0.01103	0.0104±0.00328	0.26065	0.84805	0.00274
<i>Anaerolineaceae_uncultured</i>	0±0	0±0	0.00058±0.00058	1.00000	1.00000	0.39968
<i>Anaerostipes</i>	1.29186±0.40432	0.04986±0.02226	0.01231±0.01159	0.00484	0.14894	0.00282
<i>Anaerotruncus</i>	0.77713±0.36482	1.53538±0.27758	0.81297±0.35685	0.10951	0.12560	0.94840
<i>Anaerovibrio</i>	1.03718±0.22773	1.00465±0.37017	0.78444±0.45957	0.93799	0.77328	0.70861

Table III. Continued.

Phylum/Genus	HB (%) mean \pm SD	HF (%) mean \pm SD	Ctl (%) mean \pm SD	P-value		
				HB vs. HF	HF vs. Ctl	HB vs. Ctl
<i>Anaerovorax</i>	0 \pm 0	0.01789 \pm 0.00528	0.02409 \pm 0.00572	0.00299	0.51230	0.00026
<i>Anoxybacillus</i>	0.00183 \pm 0.00095	0.00072 \pm 0.00072	0.00239 \pm 0.00176	0.65574	0.37696	0.72123
<i>Aquabacterium</i>	0.00044 \pm 0.00044	0 \pm 0	0.0013 \pm 0.0013	1.00000	0.50079	0.56788
<i>Arenimonas</i>	0 \pm 0	0 \pm 0	0.00065 \pm 0.00065	1.00000	1.00000	0.39968
<i>Bacillus</i>	0.00438 \pm 0.00295	0 \pm 0	0.00129 \pm 0.00083	0.14862	0.50079	0.33974
<i>Bacteroides</i>	11.32657 \pm 2.19329	2.86663 \pm 1.50674	0.50319 \pm 0.25937	0.00396	0.13701	0.00010
<i>Barnesiella</i>	0 \pm 0	0 \pm 0	0.00064 \pm 0.00064	1.00000	1.00000	0.39968
<i>Bifidobacterium</i>	0 \pm 0	0.00085 \pm 0.00085	0 \pm 0	0.38079	0.48016	1.00000
<i>Bilophila</i>	0.79589 \pm 0.15162	0.00085 \pm 0.00085	0.00157 \pm 0.00106	0.00046	1.00000	0.00003
<i>Blautia</i>	0.80417 \pm 0.24782	6.23756 \pm 1.76412	6.90259 \pm 2.62767	0.00509	0.86245	0.02394
<i>Brachybacterium</i>	0 \pm 0	0 \pm 0	0.00197 \pm 0.00197	1.00000	0.50079	0.15974
<i>Bradyrhizobium</i>	0 \pm 0	0 \pm 0	0.00065 \pm 0.00065	1.00000	1.00000	0.39968
<i>Brevundimonas</i>	0.00216 \pm 0.00113	0 \pm 0	0 \pm 0	0.16413	1.00000	0.16476
<i>Burkholderia</i>	0.01374 \pm 0.00306	0.00895 \pm 0.00321	0.00898 \pm 0.00396	0.31982	0.98550	0.42879
<i>Butyricicoccus</i>	0 \pm 0	0 \pm 0	0.00058 \pm 0.00058	1.00000	1.00000	0.39968
<i>Butyricimonas</i>	0 \pm 0	0.00602 \pm 0.00423	0.00075 \pm 0.00075	0.16249	0.23333	0.39968
<i>Candidatus_</i>	0 \pm 0	0.0005 \pm 0.0005	0.00559 \pm 0.00411	0.38079	0.23120	0.18080
<i>Arthromitus</i>						
<i>Candidatus_</i>	0.00091 \pm 0.00059	0.00135 \pm 0.00087	0 \pm 0	0.63837	0.23056	0.52013
<i>Chloracidobacterium</i>						
<i>Candidatus_</i>	0 \pm 0	0 \pm 0	0.00065 \pm 0.00065	1.00000	1.00000	0.39968
<i>Solibacter</i>						
<i>Caulobacter</i>	0.00044 \pm 0.00044	0 \pm 0	0 \pm 0	1.00000	1.00000	1.00000
<i>Cellulosilyticum</i>	0.00048 \pm 0.00048	0 \pm 0	0 \pm 0	1.00000	1.00000	1.00000
<i>Christensenellaceae_</i>	0 \pm 0	0.50197 \pm 0.122	1.05035 \pm 0.29907	0.00139	0.10517	0.00119
<i>uncultured</i>						
<i>Christensenella</i>	0 \pm 0	0.00765 \pm 0.00241	0.07967 \pm 0.01597	0.00398	0.00051	0.00007
<i>Chryseobacterium</i>	0.00136 \pm 0.00066	0 \pm 0	0 \pm 0	0.29263	1.00000	0.28019
<i>Clostridium</i>	1.0162 \pm 0.90173	0.00465 \pm 0.00191	0.0035 \pm 0.00288	0.29069	0.79322	0.27462
<i>Collinsella</i>	0 \pm 0	1.75193 \pm 0.70971	6.94762 \pm 2.43052	0.02134	0.05349	0.00609
<i>Comamonas</i>	0.00588 \pm 0.00213	0.00229 \pm 0.00148	0.00369 \pm 0.00194	0.18018	0.51090	0.54084
<i>Coprococcus</i>	0.00187 \pm 0.00101	0.34846 \pm 0.07673	0.44409 \pm 0.23277	0.00092	0.76015	0.06288
<i>Coriobacteriaceae_</i>	0.00044 \pm 0.00044	0.07323 \pm 0.01542	0.07895 \pm 0.02065	0.00080	0.85713	0.00067
<i>uncultured</i>						
<i>Corynebacterium</i>	0 \pm 0	0.00751 \pm 0.00291	0.00305 \pm 0.00119	0.01717	0.17098	0.01020
<i>Deinococcus</i>	0.00137 \pm 0.00095	0.00085 \pm 0.00085	0.00065 \pm 0.00065	1.00000	1.00000	1.00000
<i>Delftia</i>	0.02537 \pm 0.00587	0.00866 \pm 0.00411	0.01068 \pm 0.00254	0.02783	0.74368	0.02491
<i>Desemzia</i>	0.00048 \pm 0.00048	0 \pm 0	0 \pm 0	1.00000	1.00000	1.00000
<i>Desulfovibrio</i>	0.13322 \pm 0.0136	2.12679 \pm 0.44718	1.30345 \pm 0.47798	0.00099	0.22106	0.01686
<i>Devosia</i>	0.00092 \pm 0.0006	0 \pm 0	0 \pm 0	0.52842	1.00000	0.52013
<i>Diaphorobacter</i>	0.00049 \pm 0.00049	0 \pm 0	0.00117 \pm 0.00117	1.00000	0.50079	0.56788
<i>Elusimicrobium</i>	0 \pm 0	0.00207 \pm 0.00089	0.00232 \pm 0.00114	0.05521	1.00000	0.06384
<i>Enhydrobacter</i>	0.00084 \pm 0.00055	0 \pm 0	0 \pm 0	0.52842	1.00000	0.52013
<i>Enterococcus</i>	0.3229 \pm 0.06599	0.002 \pm 0.002	0.00649 \pm 0.00376	0.00075	0.31480	0.00011
<i>Enterorhabdus</i>	0.00043 \pm 0.00043	0.01485 \pm 0.00579	0.07087 \pm 0.0206	0.02093	0.01589	0.00164
<i>Epulopiscium</i>	0.1753 \pm 0.17421	0.00088 \pm 0.00088	0 \pm 0	0.36952	0.48016	0.34371
<i>Erysipelothrix</i>	0.00096 \pm 0.00096	0 \pm 0	0 \pm 0	0.52842	1.00000	0.52013
<i>Erysipelotrichaceae_</i>	2.50531 \pm 0.39665	0.00672 \pm 0.00292	0.01301 \pm 0.00902	0.00017	0.58704	0.00002
<i>Incertae_Sedis</i>						

Table III. Continued.

Phylum/Genus	HB (%) mean \pm SD	HF (%) mean \pm SD	Ctl (%) mean \pm SD	P-value		
				HB vs. HF	HF vs. Ctl	HB vs. Ctl
<i>Erysipelotrichaceae_ uncultured</i>	0 \pm 0	0.00648 \pm 0.00587	0.01533 \pm 0.00785	0.30366	0.45472	0.05456
<i>Escherichia-Shigella</i>	0.35447 \pm 0.11707	0.02743 \pm 0.00973	0.32604 \pm 0.1537	0.01092	0.07005	0.90572
<i>Faecalibacterium</i>	0.00173 \pm 0.00131	0.81438 \pm 0.32885	0.00058 \pm 0.00058	0.02116	0.02256	0.65440
<i>Family_XIII_ Incertae_Sedis_ Incertae_Sedis</i>	0 \pm 0	0.10057 \pm 0.02552	0.20788 \pm 0.04714	0.00163	0.06129	0.00018
<i>Family_XIII_ Incertae_Sedis_ uncultured</i>	0 \pm 0	0.36272 \pm 0.09627	0.21403 \pm 0.05279	0.00193	0.19001	0.00036
<i>Ferruginibacter</i>	0.0008 \pm 0.0008	0 \pm 0	0.00058 \pm 0.00058	0.52842	1.00000	1.00000
<i>Flavobacterium</i>	0.00096 \pm 0.00096	0.00264 \pm 0.00264	0.00058 \pm 0.00058	0.37584	0.35637	1.00000
<i>Flavonifractor</i>	0.01386 \pm 0.01386	0.00451 \pm 0.00451	0.0693 \pm 0.05199	0.61083	0.22530	0.32534
<i>Flexibacter</i>	0.00048 \pm 0.00048	0 \pm 0	0.0013 \pm 0.0013	1.00000	0.50079	0.56788
<i>Fusobacterium</i>	40.17087 \pm 4.4684	0.03271 \pm 0.01065	0.01066 \pm 0.00678	0.00004	0.09584	0.00000
<i>GKS98_freshwater_ group</i>	0.00088 \pm 0.00088	0 \pm 0	0 \pm 0	0.52842	1.00000	0.52013
<i>Gelria</i>	0 \pm 0	0.0005 \pm 0.0005	0 \pm 0	0.38079	0.48016	1.00000
<i>Gemella</i>	0.00776 \pm 0.00299	0.16081 \pm 0.06141	0.00508 \pm 0.00187	0.02078	0.01988	0.53684
<i>Gemmatimonadaceae_ uncultured</i>	0.00095 \pm 0.00095	0 \pm 0	0 \pm 0	0.52842	1.00000	0.52013
<i>Geobacillus</i>	0.00087 \pm 0.00057	0 \pm 0	0 \pm 0	0.52842	1.00000	0.52013
<i>Globicatella</i>	0 \pm 0	0 \pm 0	0.00129 \pm 0.00083	1.00000	0.50079	0.15974
<i>Granulicatella</i>	0 \pm 0	0.00088 \pm 0.00088	0 \pm 0	0.38079	0.48016	1.00000
<i>Haliangium</i>	0.00048 \pm 0.00048	0 \pm 0	0 \pm 0	1.00000	1.00000	1.00000
<i>Helicobacter</i>	0.07094 \pm 0.02612	0.03422 \pm 0.01028	0.01257 \pm 0.006	0.20991	0.08585	0.03429
<i>Herbaspirillum</i>	0 \pm 0	0.00144 \pm 0.00144	0 \pm 0	0.14500	0.23056	1.00000
<i>Holdemania</i>	0 \pm 0	0.06632 \pm 0.01785	0.09885 \pm 0.0582	0.00208	0.66766	0.09437
<i>Hydrogenoanaerobacterium</i>	0 \pm 0	0.0005 \pm 0.0005	0 \pm 0	0.38079	0.48016	1.00000
<i>Hydrogenophaga</i>	0 \pm 0	0.00072 \pm 0.00072	0.00065 \pm 0.00065	0.38079	1.00000	0.39968
<i>Iamia</i>	0.00048 \pm 0.00048	0 \pm 0	0 \pm 0	1.00000	1.00000	1.00000
<i>Janthinobacterium</i>	0.0004 \pm 0.0004	0 \pm 0	0 \pm 0	1.00000	1.00000	1.00000
<i>Jeotgalicoccus</i>	0.00044 \pm 0.00044	0 \pm 0	0 \pm 0	1.00000	1.00000	1.00000
<i>Klebsiella</i>	2.78132 \pm 0.76059	0.0005 \pm 0.0005	0.00122 \pm 0.00079	0.00220	1.00000	0.00088
<i>Kocuria</i>	0.00654 \pm 0.00483	0 \pm 0	0 \pm 0	0.19016	1.00000	0.18444
<i>Lachnospiraceae_ Incertae_Sedis</i>	3.56313 \pm 0.69197	1.58603 \pm 0.57302	1.85636 \pm 0.36867	0.03981	0.75605	0.03432
<i>Lachnospiraceae_ uncultured</i>	0.01129 \pm 0.00569	6.64096 \pm 2.13871	10.66917 \pm 3.4409	0.00469	0.39685	0.00338
<i>Lachnospira</i>	0 \pm 0	0.00171 \pm 0.00171	0.0047 \pm 0.00389	0.14500	0.45570	0.01020
<i>Lactobacillus</i>	0.69316 \pm 0.42232	3.77028 \pm 2.08403	16.53313 \pm 5.33947	0.15694	0.03801	0.00458
<i>Lactococcus</i>	0.0058 \pm 0.00206	0.00072 \pm 0.00072	0 \pm 0	0.02900	0.48016	0.00690
<i>Leifsonia</i>	0 \pm 0	0 \pm 0	0.00065 \pm 0.00065	1.00000	1.00000	0.39968
<i>Leptolyngbya</i>	0 \pm 0	0 \pm 0	0.00254 \pm 0.00194	1.00000	0.12618	0.02552
<i>Leptothrix</i>	0.00043 \pm 0.00043	0 \pm 0	0 \pm 0	1.00000	1.00000	1.00000
<i>Leucobacter</i>	0.00044 \pm 0.00044	0.00088 \pm 0.00088	0 \pm 0	1.00000	0.48016	1.00000
<i>Leuconostoc</i>	0.00314 \pm 0.00103	0 \pm 0	0 \pm 0	0.04928	1.00000	0.04686
<i>Luteimonas</i>	0 \pm 0	0 \pm 0	0.00099 \pm 0.00099	1.00000	1.00000	0.39968
<i>Lysobacter</i>	0 \pm 0	0 \pm 0	0.00058 \pm 0.00058	1.00000	1.00000	0.39968

Table III. Continued.

Phylum/Genus	HB (%) mean ± SD	HF (%) mean ± SD	Ctl (%) mean ± SD	P-value		
				HB vs. HF	HF vs. Ctl	HB vs. Ctl
<i>Marmoricola</i>	0.00048±0.00048	0±0	0±0	1.00000	1.00000	1.00000
<i>Marvinbryantia</i>	0±0	0.15705±0.04201	0.13174±0.03237	0.00203	0.70828	0.00033
<i>Megamonas</i>	0±0	0±0	0.00058±0.00058	1.00000	1.00000	0.39968
<i>Methylobacillus</i>	0±0	0±0	0.00065±0.00065	1.00000	1.00000	0.39968
<i>Methylobacterium</i>	0.00084±0.00055	0±0	0.00117±0.00117	0.52842	0.50079	0.65412
<i>Microbacterium</i>	0±0	0.00176±0.00176	0±0	0.14500	0.23056	1.00000
<i>Micrococcus</i>	0.0004±0.0004	0±0	0±0	1.00000	1.00000	1.00000
<i>Mogibacterium</i>	0±0	0.10606±0.02317	0.06192±0.03252	0.00090	0.28500	0.06173
<i>Morganella</i>	0.63669±0.10501	0.00122±0.00077	0±0	0.00024	0.23056	0.00002
<i>Mucilaginibacter</i>	0±0	0±0	0.0013±0.0013	1.00000	0.50079	0.15974
<i>Mucispirillum</i>	0±0	0.00314±0.00194	0.00756±0.00683	0.02102	0.61466	0.28153
<i>Mycobacterium</i>	0.00088±0.00058	0±0	0±0	0.52842	1.00000	0.52013
<i>Mycoplasma</i>	0.013±0.01151	0±0	0.00075±0.00075	0.28651	1.00000	0.30851
<i>Nesterenkonia</i>	0±0	0±0	0.00064±0.00064	1.00000	1.00000	0.39968
<i>Nitrospiraceae_</i> <i>uncultured</i>	0±0	0.00072±0.00072	0±0	0.38079	0.48016	1.00000
<i>Ochrobactrum</i>	0.01515±0.00334	0.00684±0.00496	0.00507±0.00217	0.17780	0.79573	0.01367
<i>Odoribacter</i>	0.02862±0.01189	0.0005±0.0005	0.00304±0.00111	0.02645	0.37696	0.03689
<i>Opitutus</i>	0±0	0.00072±0.00072	0.00198±0.00094	0.38079	0.62618	0.06384
<i>Oscillibacter</i>	0.00134±0.00092	1.91854±0.4873	0.47583±0.15936	0.00169	0.01063	0.00438
<i>Oscillospira</i>	0.00043±0.00043	0.1396±0.06182	0.05487±0.0542	0.03471	0.32838	0.34728
<i>Ottowia</i>	0±0	0±0	0.00409±0.00409	1.00000	0.34499	0.35432
<i>Oxalobacter</i>	0±0	0.04147±0.01443	0±0	0.00911	0.00915	1.00000
<i>Paenisporosarcina</i>	0±0	0±0	0.00233±0.00233	1.00000	0.12618	0.02552
<i>Papillibacter</i>	0±0	0.00885±0.00248	0.01761±0.00523	0.00235	0.14454	0.00181
<i>Parabacteroides</i>	0.00087±0.00057	0.29747±0.10585	0.76933±0.24052	0.01072	0.08878	0.00256
<i>Pasteurella</i>	0.0104±0.00628	0.00144±0.00144	0.00442±0.00327	0.17627	0.49073	0.49161
<i>Paucimonas</i>	0±0	0.00072±0.00072	0±0	0.38079	0.48016	1.00000
<i>Pelomonas</i>	0.00266±0.00114	0±0	0.00058±0.00058	0.08916	1.00000	0.25499
<i>Peptococcaceae_</i> <i>uncultured</i>	0±0	0.11683±0.04023	0.05492±0.01607	0.00863	0.16894	0.00164
<i>Peptococcus</i>	0±0	0.01105±0.00382	0.01071±0.00654	0.00879	0.96040	0.10656
<i>Peptostreptococ-</i> <i>aceae_Incertae_Sedis</i>	9.05387±0.70459	0.05952±0.03087	0.11667±0.03346	0.00000	0.22173	0.00000
<i>Peptostreptococcus</i>	0.00048±0.00048	0±0	0±0	1.00000	1.00000	1.00000
<i>Phascolarcto-</i> <i>bacterium</i>	4.99382±0.69833	3.28502±1.54339	0.532±0.40397	0.36342	0.09938	0.00003
<i>Phyllobacterium</i>	0.00188±0.00101	0±0	0±0	0.30479	1.00000	0.15540
<i>Pir4_lineage</i>	0.00044±0.00044	0±0	0±0	1.00000	1.00000	1.00000
<i>Prevotellaceae_</i> <i>uncultured</i>	0±0	0.64183±0.16969	0.9912±0.41729	0.00190	0.52234	0.02007
<i>Prevotella</i>	0.00545±0.00269	1.28652±0.75418	0.93156±0.2499	0.10279	0.72772	0.00083
<i>Propionibacterium</i>	0.00362±0.00157	0.004±0.00177	0.00122±0.00079	0.89485	0.16840	0.17603
<i>Proteiniphilum</i>	0±0	0±0	0.00467±0.00467	1.00000	0.34499	0.35432
<i>Proteus</i>	0.03882±0.00637	0±0	0±0	0.00021	1.00000	0.00002
<i>Pseudomonas</i>	0±0	0±0	0.00376±0.00376	1.00000	0.06348	0.01020
<i>Pseudorhodoferrax</i>	0.0008±0.0008	0±0	0±0	0.52842	1.00000	0.52013
<i>Pseudoxanthomonas</i>	0.00048±0.00048	0.002±0.002	0±0	0.07310	0.05316	1.00000
<i>RC9_gut_group</i>	0±0	0.2138±0.0391	0.25933±0.08393	0.00037	0.69826	0.00352
<i>Ramlibacter</i>	0±0	0.00216±0.00216	0±0	0.05521	0.11070	1.00000

Table III. Continued.

Phylum/Genus	HB (%) mean \pm SD	HF (%) mean \pm SD	Ctl (%) mean \pm SD	P-value		
				HB vs. HF	HF vs. Ctl	HB vs. Ctl
<i>Rhodobacteraceae_</i> <i>uncultured</i>	0.00044 \pm 0.00044	0 \pm 0	0 \pm 0	1.00000	1.00000	1.00000
<i>Rhodococcus</i>	0.00048 \pm 0.00048	0 \pm 0	0.00058 \pm 0.00058	1.00000	1.00000	1.00000
<i>Rhodocytophaga</i>	0.00086 \pm 0.00086	0 \pm 0	0 \pm 0	0.52842	1.00000	0.52013
<i>Rikenella</i>	0 \pm 0	0 \pm 0	0.00175 \pm 0.00175	1.00000	0.25118	0.06384
<i>Robinsoniella</i>	1.60965 \pm 0.60768	0.00171 \pm 0.00171	0.00075 \pm 0.00075	0.01464	0.61074	0.01013
<i>Roseburia</i>	0.00043 \pm 0.00043	3.29178 \pm 0.50767	0.72816 \pm 0.24779	0.00015	0.00045	0.00493
<i>Roseomonas</i>	0 \pm 0	0.0005 \pm 0.0005	0 \pm 0	0.38079	0.48016	1.00000
<i>Rothia</i>	0.00083 \pm 0.00055	0.02233 \pm 0.00892	0.01708 \pm 0.00747	0.02418	0.72530	0.03511
<i>Ruminococcaceae_</i> <i>Incertae_Sedis</i>	0.53333 \pm 0.18071	1.4356 \pm 0.21452	0.98897 \pm 0.17647	0.00379	0.12410	0.07627
<i>Ruminococcaceae_</i> <i>uncultured</i>	1.58302 \pm 0.3162	13.47235 \pm 2.72614	8.78594 \pm 2.20227	0.00104	0.19671	0.00236
<i>Ruminococcus</i>	0 \pm 0	0.40738 \pm 0.06601	0.70982 \pm 0.1478	0.00019	0.07870	0.00011
<i>Saccharopolyspora</i>	0.00048 \pm 0.00048	0 \pm 0	0 \pm 0	1.00000	1.00000	1.00000
<i>Salinicoccus</i>	0 \pm 0	0 \pm 0	0.00064 \pm 0.00064	1.00000	1.00000	0.39968
<i>Saprospiraceae_</i> <i>uncultured</i>	0 \pm 0	0.00144 \pm 0.00144	0 \pm 0	0.14500	0.23056	1.00000
<i>Selenomonas</i>	0 \pm 0	0.01814 \pm 0.01216	0.01602 \pm 0.00427	0.14733	0.88951	0.00082
<i>Sinobacteraceae_</i> <i>uncultured</i>	0 \pm 0	0.00072 \pm 0.00072	0.00075 \pm 0.00075	0.38079	1.00000	0.39968
<i>Sphingobacteriaceae_</i> <i>uncultured</i>	0 \pm 0	0 \pm 0	0.00058 \pm 0.00058	1.00000	1.00000	0.39968
<i>Sphingobium</i>	0.00043 \pm 0.00043	0 \pm 0	0 \pm 0	1.00000	1.00000	1.00000
<i>Sphingomonas</i>	0.00253 \pm 0.00159	0.00232 \pm 0.00149	0 \pm 0	1.00000	0.11070	0.08762
<i>Sphingopyxis</i>	0.00049 \pm 0.00049	0 \pm 0	0 \pm 0	1.00000	1.00000	1.00000
<i>Staphylococcus</i>	0.00695 \pm 0.0036	0.01002 \pm 0.00518	0.0181 \pm 0.00654	0.69869	0.41635	0.13924
<i>Stenotrophomonas</i>	0.00402 \pm 0.00308	0.00144 \pm 0.00144	0.00637 \pm 0.00439	0.53373	0.30516	0.73974
<i>Streptococcus</i>	0.34317 \pm 0.15446	0.85093 \pm 0.48484	0.13616 \pm 0.05995	0.37540	0.16071	0.22702
<i>Streptomyces</i>	0 \pm 0	0.00088 \pm 0.00088	0 \pm 0	0.38079	0.48016	1.00000
<i>Subdoligranulum</i>	0 \pm 0	0.0005 \pm 0.0005	0 \pm 0	0.38079	0.48016	1.00000
<i>Sutterella</i>	0.0004 \pm 0.0004	0.04729 \pm 0.01319	0.00962 \pm 0.00278	0.00254	0.01109	0.00213
<i>Syntrophobacter</i> <i>aceae_uncultured</i>	0.0008 \pm 0.0008	0 \pm 0	0 \pm 0	0.52842	1.00000	0.52013
<i>Tetragenococcus</i>	0 \pm 0	0 \pm 0	0.00186 \pm 0.00131	1.00000	0.25118	0.06384
<i>Thalassolituus</i>	0.0004 \pm 0.0004	0 \pm 0	0 \pm 0	1.00000	1.00000	1.00000
<i>Thalassospira</i>	0.00048 \pm 0.00048	0.01421 \pm 0.00381	0.02303 \pm 0.00768	0.00233	0.32926	0.00499
<i>Thauera</i>	0 \pm 0	0.0005 \pm 0.0005	0.00175 \pm 0.00175	0.38079	0.62618	0.06384
<i>Thiobacillus</i>	0 \pm 0	0 \pm 0	0.00759 \pm 0.00759	1.00000	0.34499	0.35432
<i>Thiotrichaceae_</i> <i>uncultured</i>	0 \pm 0	0 \pm 0	0.00075 \pm 0.00075	1.00000	1.00000	0.39968
<i>Treponema</i>	0.0013 \pm 0.0013	0.89427 \pm 0.16598	0.20351 \pm 0.11878	0.00044	0.00327	0.09380
<i>Trichococcus</i>	0 \pm 0	0.00085 \pm 0.00085	0 \pm 0	0.38079	0.48016	1.00000
<i>Turicibacter</i>	0.02804 \pm 0.01479	0.00144 \pm 0.00144	0.00824 \pm 0.0025	0.08861	0.02923	0.20080
<i>Veillonella</i>	0.00347 \pm 0.00128	0.00594 \pm 0.0033	0.00065 \pm 0.00065	0.57852	0.13088	0.05251
<i>Victivallis</i>	0 \pm 0	0.0088 \pm 0.00401	0.00389 \pm 0.00251	0.04043	0.32513	0.00408
<i>Xanthobacteraceae_</i> <i>uncultured</i>	0 \pm 0	0 \pm 0	0.00065 \pm 0.00065	1.00000	1.00000	0.39968
<i>Xylanibacter</i>	0 \pm 0	0.22435 \pm 0.186	0.01461 \pm 0.00458	0.25461	0.27539	0.00258

Table III. Continued.

Phylum/Genus	HB (%) mean \pm SD	HF (%) mean \pm SD	Ctl (%) mean \pm SD	P-value		
				HB vs. HF	HF vs. Ctl	HB vs. Ctl
<i>vadinBC27_waste</i> <i>water-sludge_group</i>	0 \pm 0	0 \pm 0	0.00075 \pm 0.00075	1.00000	1.00000	0.39968

Difference in the relative abundance of phylotypes defined at 97%-ID in rats in the Ctl, HF and HB groups. Ctl, normal diet; HF, HFD; HB, HFD supplemented with berberine for 6 weeks; HFD, high-fat diet.

gut microbiome changed along the first ordination axis, which accounted for 72.8% of total variance (Fig. 9F).

Berberine appeared to significantly alter the abundance of 34 genera, 14 of which were increased, whereas the remaining 20 were decreased or eliminated (Table IV). Notably, the genus *Akkermansia* of the *Verrucomicrobia* phylum could not be detected in the HF group however, it was detected in the HB group. The genera *Collinsella*, *Prevotellaceae_uncultured*, *Christensenellaceae_uncultured* and *Ruminococcus* were detected in the HF group however, not in the HB group. Furthermore, the relative abundance of 21 genera appeared significantly increased in the HF group compared with in the control group. Among these, treatment with berberine significantly decreased the abundance of 11 genera, including *Roseburia*, *Allobaculum*, *Oscillibacter*, *Faecalibacterium*, *Prevotella* and *Desulfovibrio*. A total of 3 genera (*Coprococcus*, *Collinsella* and *Blautia*) remained unaffected by the HFD, however, they were significantly decreased following berberine supplementation. In addition, 10 genera appeared significantly decreased in the HFD-fed group, of which 4 were significantly increased following treatment with berberine, including *Erysipelotrichaceae_Incertae_Sedis*, *Peptostreptococcaceae_Incertae_Sedis* and *Escherichia-Shigella*. Furthermore, the genera *Fusobacterium*, *Anaerostipes*, *Bacteroides* and *Phascolarctobacterium* were also significantly increased by treatment with berberine.

In order to identify the specific genera of intestinal bacteria that could be associated with the beneficial effects of berberine, Spearman's correlation analysis was performed between the 34 genera whose distribution appeared to be altered following berberine supplementation and a number of physiological parameters. The analyses revealed that weight, glucose intolerance, FITC-dextran area and L-cell number were correlated with the abundance of several genera (Table V).

Discussion

It has previously been demonstrated that berberine participates in the regulation of glucose and lipid metabolism through targeting gut microbiota; however, the implication of its modulatory effects on gut microbiota in metabolic disorders has not yet been examined. Growing evidence has suggested that the gut microbiome contributes to the systemic low-grade inflammation that is characteristic of metabolic disorders. LPS from intestinal bacteria can leak into the

circulation through the damaged intestinal mucosa (15), where it can cause metabolic endotoxemia and the production of proinflammatory cytokines, thereby contributing to insulin resistance and related metabolic disorders (26,27). Plasma LPS levels are a direct biomarker of systemic inflammation. The present study revealed that berberine can significantly decrease plasma LPS levels, which is in accordance with previous results reporting that berberine can significantly prevent HFD-induced systemic inflammation by decreasing serum LPS-binding protein levels (14). Since macrophage infiltration and oxidative stress in adipose tissue participate in inflammation and insulin resistance (28-30), a number of inflammatory and oxidative stress factors were investigated in order to evaluate the role of berberine in systemic inflammation. Berberine appeared to reduce the HFD-induced mRNA expression levels of markers of inflammation (IL-1 β and PAI-1), oxidative stress (NADPHox and STAMP-2) and macrophage infiltration (MCP-1 and F4/80) in visceral adipose tissue. Berberine has previously been reported to downregulate the expression of the proinflammatory cytokines TNF- α , IL-1, IL-6 and MCP-1 in white adipose tissue from db/db mice (31). Furthermore, the correlations that were revealed in the present study between these markers and plasma LPS levels further support the hypothesis that berberine can improve the endotoxemia-induced systemic inflammation in HFD-fed rats.

The present results suggested that the beneficial effect of berberine on endotoxemia is unlikely to be a result of a decrease in Gram-negative bacteria (Table III), thus indicating that berberine may reduce endotoxemia through reducing intestinal permeability. Alterations in intestinal permeability have previously been associated with alterations in the expression, localization and distribution of tight junction proteins, including claudins, ZO-1 and occludin (32). It has also been suggested that berberine may directly affect the expression of tight junction proteins. Amasheh *et al* (33) reported that in HT-29/B6 cells, berberine increased the mRNA expression levels of claudin-1, however, not claudin-2. In a rat model of LPS-induced injury, berberine administration following LPS injection did not appear to ameliorate the expression and distribution of the tight junction proteins claudin-1, claudin-4, ZO-1 and occludin, and had no effect on intestinal permeability. However, pretreatment with berberine for 7 days was reported to partially attenuate the LPS-induced destruction and redistribution of tight junction proteins (34). Considering the pharmacokinetic features of berberine, it may be hypothesized

Table IV. Differences of the 34 genera identified by RDA between HB and HF groups.

Genus	Genus for short	Phylum	Class	Order	Family	HB (%)	HF (%)	Change (%)
<i>Akkermansia</i>	Akke_man	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	3.14836±1.01832	0.00000±0.00000	HB ^a
<i>Anaeroflum</i>	Anae_fil	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	2.30376±0.73263	0.05499±0.02737	4089.2
<i>Anaerostipes</i>	Anae_sti	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	1.29186±0.40432	0.04986±0.02226	2490.8
<i>Bacteroides</i>	Bact_oid	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	11.32657±2.19329	2.86663±1.50674	295.1
<i>Bilophila</i>	Bilo_phi	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	0.79589±0.15162	0.00085±0.00085	93122.1
<i>Erysipelotrichaceae</i>	Erys_Sed	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	2.50531±0.39665	0.00672±0.00292	37190.7
<i>Incertae_Sedis</i>								
<i>Escherichia-Shigella</i>	Esch_Shi	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	0.35447±0.11707	0.02743±0.00973	1192.4
<i>Fusobacterium</i>	Fuso_bac	Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	40.17087±4.46840	0.03271±0.01065	122701.9
<i>Klebsiella</i>	Kleb_sie	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	2.78132±0.76059	0.00050±0.00050	555400.0
<i>Lachnospiraceae</i>	Lach_Sed	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	3.56313±0.69197	1.58603±0.57302	124.7
<i>Incertae_Sedis</i>								
<i>Morganella</i>	Morg_nel	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	0.63669±0.10501	0.00122±0.00077	52081.4
<i>Peptostreptococcaceae</i>	Pept_Sed	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	9.05387±0.70459	0.05952±0.03087	15110.5
<i>Incertae_Sedis</i>								
<i>Phascolarctobacterium</i>	Phas_bac	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	4.99382±0.69833	3.28502±1.54339	52.0
<i>Robinsoniella</i>	Robi_son	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	1.60965±0.60768	0.00171±0.00171	94285.6
<i>Allobaculum</i>	Allo_bac	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	0.00311±0.00079	10.84206±3.94134	-100.0
<i>Anaerotruncus</i>	Anae_tru	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.77713±0.36482	1.53538±0.27758	-49.4
<i>Blautia</i>	Blautia	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	0.80417±0.24782	6.23756±1.76412	-87.1
<i>Christensenellaceae</i>	Chri_unc	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	0.00000±0.00000	0.50197±0.12200	HF ^b
<i>uncultured</i>								
<i>Collinsella</i>	Coll_sel	Actinobacteria	Actinobacteria	Coriobacteriales	Coriobacteriaceae	0.00000±0.00000	1.75193±0.70971	HF ^b
<i>Coproccoccus</i>	Copr_coc	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	0.00187±0.00101	0.34846±0.07673	-99.5
<i>Desulfovibrio</i>	Desu_vib	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	0.13322±0.01360	2.12679±0.44718	-93.7
<i>Faecalibacterium</i>	Faec_bac	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.00173±0.00131	0.81438±0.32885	-99.8
<i>Lachnospiraceae</i>	Lach_unc	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	0.01129±0.00569	6.64096±2.13871	-99.8
<i>uncultured</i>								
<i>Lactobacillus</i>	Lact_bac	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	0.69316±0.42232	3.77028±2.08403	-81.6
<i>Oscillibacter</i>	Osci_bac	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.00134±0.00092	1.91854±0.48730	-99.9
<i>Parabacteroides</i>	Para_bac	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	0.00087±0.00057	0.29747±0.10585	-99.7
<i>Prevotella</i>	Prev_tel	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	0.00545±0.00269	1.28652±0.75418	-99.6
<i>Prevotellaceae</i>	Prev_unc	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	0.00000±0.00000	0.64183±0.16969	HF ^b
<i>uncultured</i>								

Table IV. Continued.

Genus	Genus for short	Phylum	Class	Order	Family	HB (%)	HF (%)	Change (%)
<i>Roseburia</i>	Rose_bur	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	0.00043±0.00043	3.29178±0.50767	-100.0
<i>Ruminococcaceae_Incertae_Sedis</i>	Rumi_Sed	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.53333±0.18071	1.43560±0.21452	-62.8
<i>Ruminococcaceae_uncultured</i>	Rumi_unc	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	1.58302±0.31620	13.47235±2.72614	-88.2
<i>Ruminococcus</i>	Rumi_coc	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.00000±0.00000	0.40738±0.06601	HF ^b
<i>Streptococcus</i>	Stre_coc	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	0.34317±0.15446	0.85093±0.48484	-59.7
<i>Treponema</i>	Trep_nem	Spirochaetes	Spirochaetes(class)	Spirochaetales	Spirochaetaceae	0.00130±0.00130	0.89427±0.16598	-99.9

Data are expressed as the mean ± standard deviation. Only the percentage change values with significant differences ($P < 0.05$) between the HB and HF groups are indicated. Those that were not significant are indicated as follows: ^aHB, found only in berberine-treated rats; ^bHF, found only in the HFD-fed rats. HF, HFD; HB, HFD supplemented with berberine for 6 weeks; HFD, high-fat diet.

that berberine required a longer time-frame to reach its effective concentration. The present study demonstrated that berberine supplementation for 6 weeks significantly increased the expression of claudin-1 and ZO-1 in the proximal colon of HFD-induced obese rats. In addition, berberine appeared to partially restore the intestinal distribution of claudin-1 and claudin-2. These results suggested that berberine, through increasing the expression and restoring the distribution of tight junction proteins may contribute to the restoration of intestinal epithelial integrity.

In addition to its direct effects on the expression of tight junction proteins, berberine has been reported to indirectly restore gut permeability, through modulating gut microbiota. Previous studies have suggested that gut microbiota may regulate epithelial permeability (32,35,36). SCFAs, which are the main metabolic products of bacterial fermentation, have been suggested to improve the function of the gut barrier by promoting epithelial cell growth and facilitating tight junction formation (37,38). Zhang *et al* (14) reported that berberine, through increasing the SCFA-producing genera *Blautia* and *Allobaculum*, enhanced intestinal integrity and thus antagonized obesity. However, the results of the present study revealed that berberine significantly decreased *Blautia* and *Allobaculum* bacteria, although intestinal permeability was improved. The present results agree with a previous report by Xie *et al* (2) demonstrating that berberine exerted anti-obesity effects partly by decreasing the degradation of dietary polysaccharides and fecal SCFA production to inhibit energy harvest. Previous studies have suggested that the relationship between fecal SCFAs and the regulation of host metabolism is important and complex (2,16). In contrast to the model used by Zhang *et al* (14), the present study evaluated the effect of berberine in rats maintained on a HFD for 14 weeks, resembling the clinical situation. However, future studies are required to investigate the role of berberine on SCFA-producing genera of intestinal bacteria.

In the present study, a Spearman's correlation analysis revealed a negative correlation between intestinal permeability and the abundance of the *Phascolarctobacterium* and *Collinsella* genera, and berberine supplementation significantly increased the abundance of the genus *Phascolarctobacterium*. Intestinal permeability appeared positively correlated with the abundance of the *Anaerotruncus* and *Oscillibacter* genera (Table V). The present findings indicated that *Phascolarctobacterium*, *Anaerotruncus* and *Oscillibacter* may be solely responsible for the beneficial effects of berberine on intestinal permeability. A significant 10-fold decrease in the genus *Oscillibacter* was observed in berberine-treated HFD-fed rats. It has previously been reported that a HFD significantly increased the abundance of *Oscillibacter*, which was negatively correlated with transepithelial resistance and ZO-1 mRNA expression levels in the proximal colon (39). In accordance with the previous study, the present results suggested that berberine may increase ZO-1 mRNA expression levels and intestinal permeability, possibly by inhibiting *Oscillibacter* abundance. In addition, the genus *Akkermansia* was reported to be present exclusively in berberine-treated rats. Previous studies have suggested that *Akkermansia muciniphila* may restore the thickness of the intestinal mucosa and counteract HFD-induced mucosal barrier dysfunction in the colon (40), whereas it has been suggested

Table V. Spearman's correlation between the specific genera altered by berberine administration according to redundancy analysis and host metabolic parameters.

	Body weight	FBG	FINS	HOMA-		AUC of OGTT	FITC-dextran area	claudin		ZO -1	occludin	L-cells	GLP		GIP	PP	PYY
				IR	OGTT			-1	-2				-1	-2			
<i>Akkermansia</i>	-0.467	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.558	0.628	ns	ns	ns	ns	ns
<i>Anaerofilum</i>	ns	ns	ns	ns	0.537	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Anaerostipes</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.56	ns	ns	ns	ns	ns
<i>Bacteroides</i>	ns	ns	ns	ns	ns	ns	0.436	ns	ns	ns	ns	ns	0.54	ns	ns	ns	ns
<i>Bifidophila</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.558	ns	ns	ns	ns	ns
<i>Escherichia-Shigella</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Fusobacterium</i>	ns	ns	ns	ns	ns	ns	0.595	ns	ns	ns	ns	ns	0.796	0.524	ns	ns	ns
<i>Klebsiella</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Lachnospiraceae</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Incertae_Sedis</i>																	
<i>Morganella</i>	ns	ns	ns	ns	ns	ns	ns	0.582	ns	0.507	0.495	ns	ns	ns	ns	ns	ns
<i>Peptostreptococcaceae</i>	-0.462	ns	ns	ns	ns	ns	0.524	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Incertae_Sedis</i>																	
<i>Phascolarctobacterium</i>	ns	ns	ns	ns	ns	-0.522	ns	ns	0.483	ns	ns	ns	ns	ns	ns	ns	ns
<i>Robinsoniella</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.683	ns	ns
<i>Allobaculum</i>	ns	-0.531	ns	ns	ns	ns	ns	-0.531	ns	ns	ns	ns	ns	ns	-0.643	ns	ns
<i>Anaerotruncus</i>	0.683	ns	ns	ns	ns	0.435	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Blautia</i>	ns	-0.605	ns	ns	ns	ns	ns	-0.474	ns	ns	ns	ns	-0.52	-0.867	ns	ns	-0.496
<i>Christensenellaceae</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>_uncultured</i>																	
<i>Collinsella</i>	ns	ns	ns	ns	ns	-0.792	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Coprococcus</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Desulfovibrio</i>	ns	ns	ns	ns	ns	ns	-0.521	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Faecalibacterium</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Lachnospiraceae</i>	ns	ns	ns	ns	ns	ns	-0.524	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>_uncultured</i>																	
<i>Lactobacillus</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.541	-0.503	ns	ns	ns	ns
<i>Oscillibacter</i>	ns	0.525	0.565	0.543	0.697	0.473	ns	ns	-0.638	ns	ns	0.522	ns	ns	ns	-0.721	ns
<i>Parabacteroides</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.817
<i>Prevotella</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Prevotellaceae</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>_uncultured</i>																	
<i>Roseburia</i>	0.555	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.617	-0.738
<i>Ruminococcaceae</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Incertae_Sedis</i>																	

Table V. Continued.

	Body weight	FBG	FINS	HOMA-IR	AUC of OGTT	FITC-dextran area	claudin -1	claudin -2	ZO -1	occludin	L-cells	GLP -1	GLP -2	GIP	PP	PYY
<i>Ruminococcaceae uncultured</i>	0.441	ns	ns	ns	ns	ns	-0.541	ns	ns	ns	ns	-0.552	ns	ns	ns	ns
<i>Ruminococcus</i>	ns	ns	ns	ns	ns	ns	ns	0.602	ns	ns	ns	ns	ns	ns	ns	ns
<i>Streptococcus</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.514
<i>Treponema</i>	0.588	ns	0.714	0.686	0.78	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.753	ns

Values are Spearman correlation coefficients. Only significant correlations ($P < 0.05$) are indicated. ns, not significant ($P > 0.05$); FBG, fasting blood glucose; FINS, fasting blood insulin; HOMA-IR, homeostatic model assessment of insulin resistance; AUC, area under curve; OGTT, oral glucose tolerance test; FITC, fluorescein isothiocyanate; ZO-1, zonula occludens-1; GLP, glucagon-like peptide; GIP, gastric inhibitory polypeptide; PP, pancreatic polypeptide; PYY, peptide YY.

that this species may hold a key role in gut barrier function and metabolic inflammation (41). However, a significant correlation between the genus *Akkermansia* and intestinal permeability was not observed in the present study. Further work is required to explore the putative relationship between the abundance of *Akkermansia* bacteria and the integrity of the gut barrier.

The modulation of gut hormone levels by berberine has been reported to serve an important role in improving energy homeostasis. Previous studies have revealed that berberine increased the number of L-cells and the mRNA expression levels of proglucagon in the ileum, whereas it promoted GLP-1 secretion in normal and diabetic rats (9,10). In the present study, berberine significantly increased the portal plasma levels of GLP-1 and GLP-2, whereas it also increased the number of L-cells and the mRNA expression levels of proglucagon in the proximal colon. Multiple lines of evidence have linked gut microbiota with the enteroendocrine system, whereas SCFAs are the most studied among gut microbial metabolites (42). In the present study, Spearman's correlation analysis revealed that L-cell numbers were positively correlated with the abundance of 4 genera (*Akkermansia*, *Anaerostipes*, *Bilophila* and *Oscillibacter*) and negatively correlated with the abundance of the genus *Lactobacillus* (Table V). Previous studies reported a positive correlation between the abundance of bacteria of the *Akkermansia* genus and L-cell numbers in the colon, whereas *Akkermansia muciniphila* administration significantly increased GLP-1 release from colonic L-cells (43,44). Sequencing results of the present study revealed that the abundance of *Akkermansia* was significantly increased by berberine, although the correlation between *Akkermansia* and GLP-1 levels was not significant. Based on previous research that correlated the abundance of 10 genera with L-cell numbers (43), the present study confirmed that berberine increased the abundance of the genus *Akkermansia* and decreased the abundance of the genus *Lactobacillus*, which appeared to be associated with the increase in L-cell numbers and enteroendocrine peptide secretion from L-cells. In addition, Spearman's correlation analysis revealed plasma GIP levels to be negatively correlated with the abundance of the *Robinsoniella* genus.

The present study suggested that the wide shift in the gut microbiota composition induced by berberine may attenuate insulin resistance and related metabolic disorders in HFD-fed rats via several pathways. Firstly, berberine supplementation alleviated metabolic endotoxemia and subsequent systemic inflammation, via restoring the integrity of the gut barrier through increasing the expression and restoring the distribution of tight junction proteins. Furthermore, berberine modulated the plasma levels of gut hormones involved in glucose regulation and energy homeostasis, possibly via interfering with the composition of the gut microbiome. In conclusion, the present results suggested that berberine may be a potential therapeutic strategy for the treatment of obesity and insulin resistance. However, further study is required to delineate the mechanism of action of berberine.

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