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.

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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

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 alleviaton of inflammation via a reduction in serum LPS-binding protein and monocyte chemotactic 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 μl of blood was sampled from the tail vein, placed in ice-cold heparinized tubes and centrifuged (12,000 x *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 x *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* amebocyte 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-1β	GCTGTGGCAGCTACCTATGTCTTG	AGGTCGTCATCATCCCACGAG
PAI-1	AGTCTTTCCGACCAAGAGCA	CCAGTTTTGTCCCAAAGGAA
NADPHox	AAGTCATCCCCGCAACTGTTC	CCCGCTTCCTCATCTGCAATTC
STAMP-2	ATCCCATCAAAATTTGGCTT	CGCTGTGATTTGGAAGATTTAATAC
MCP-1	CAGATGCAGTTAATGCCCCAC	AGCCGACTCATTGGGATCAT
F4/80	CAGCTGTCTTCCCGACTTTC	TAATCAAGATTCCGGCCTTG
claudin-1	GCTGTCATCGGGGGCATAATA	CCTGGCATTGATAGGGGTCAT
claudin-2	GGACACTTATCAAGCGAG	CAGCAATGGGATTTAGACT
occludin	CCTCTGACCTTGTCCGTGGATG	TCCCTGCTTTCCCCTTCGTG
ZO-1	CTACCTTATTGAATGTC	AACTGAATGGTCTGATGCT
proglucogan	CCTCTATGCCAACACAGT	AGCCACCAATCCACACAG
β-actin	GGCTGGATTGTTTGTAATGC	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 form 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 ($\Delta\Delta$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

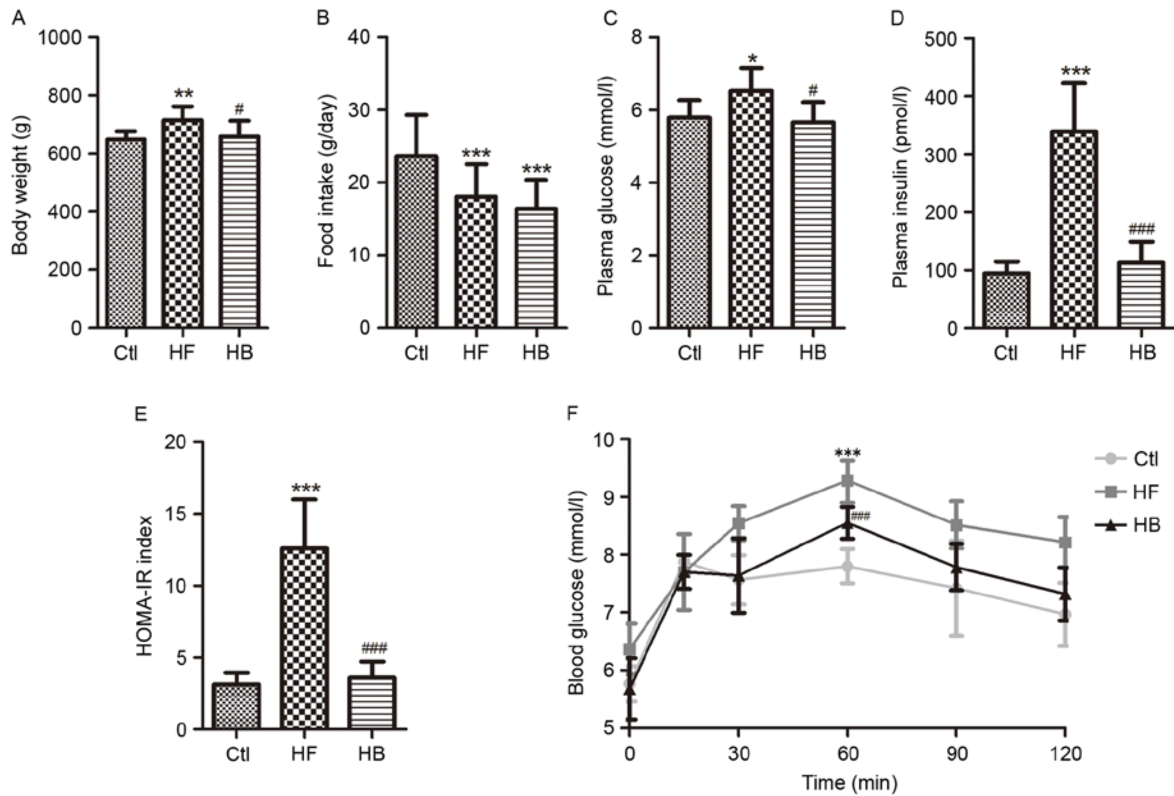

Figure 1. Berberine reduced weight gain and improved glucose homeostasis in HFD-fed rats. (A) Body weight. (B) Daily food intake per rat. (C) Fasting plasma glucose concentration. (D) Fasting plasma insulin concentration. (E) HOMA-IR index, calculated using the following equation: FBG (mmol/l) x FINS (mU/l)/22.5. (F) Oral glucose tolerance test. Data are expressed as the mean ± 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.

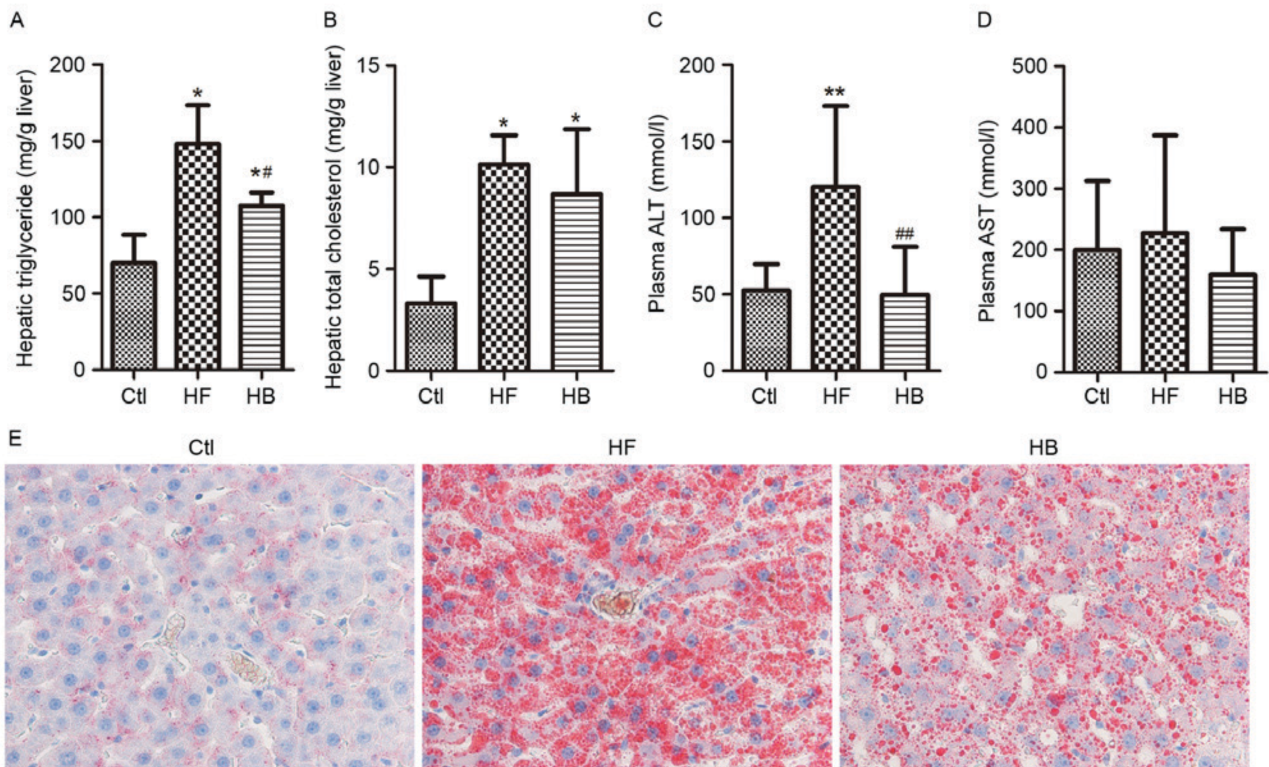

Figure 2. Berberine attenuated HFD-induced hepatic injury and hepatic steatosis. (A) Hepatic triglyceride content. (B) Hepatic total cholesterol content. (C) ALT plasma concentration. (D) AST plasma concentration. (E) Fresh-frozen liver sections were stained with Oil Red O (magnification, x100). Data are expressed as the mean ± 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'-NNNNNNNNAGAGTTTGATCCTGGCTCAG-3' and reverse primer 5'-NNNNNNNNTTACCGCGGCTGCTGGCAC-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 cycler (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 ± 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 coefficent. 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-1β (IL-1β),

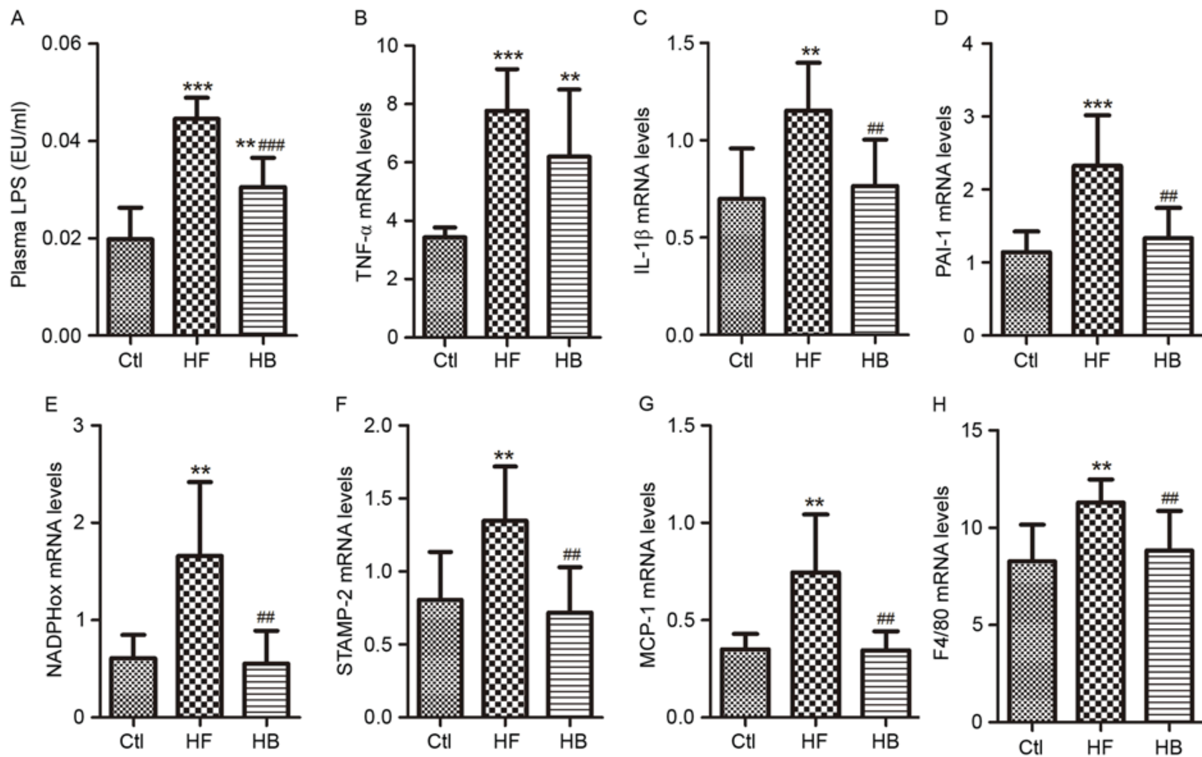

Figure 3. Berberine reduced endotoxemia and visceral adipose tissue inflammation in HFD-fed rats. (A) Portal plasma LPS concentrations. (B) TNF-α mRNA expression levels. (C) IL-1β mRNA expression levels. (D) PAI-1 mRNA expression levels. (E) NADPHox mRNA expression levels. (F) STAMP-2 mRNA expression levels. (G) MCP-1 mRNA expression levels. (H) F4/80 mRNA expression levels. Data are expressed as the mean ± 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 chemotactic protein-1; F4/80, EGF-like module-containing mucin-like hormone receptor-like 1.

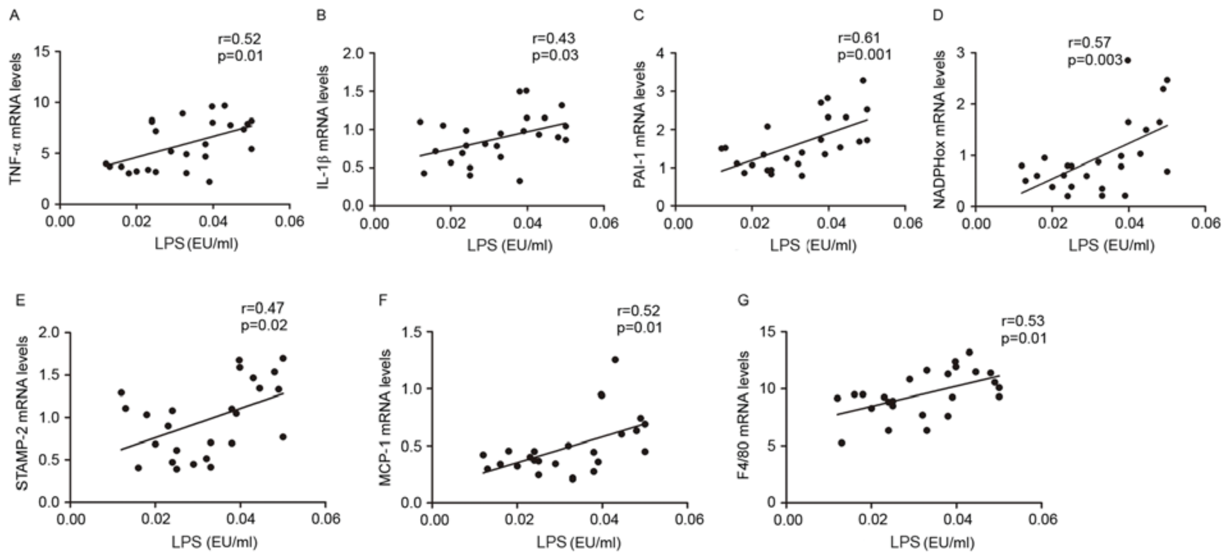

Figure 4. Metabolic endotoxemia was positively correlated with inflammation, oxidative stress and macrophage infiltration markers. Correlation between: Portal plasma LPS level and (A) TNF-α, (B) IL-1β, (C) PAI-1, (D) NADPHox, (E) STAMP-2, (F) MCP-1 and (G) F4/80 mRNA levels in visceral adipose tissue 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 chemotactic 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

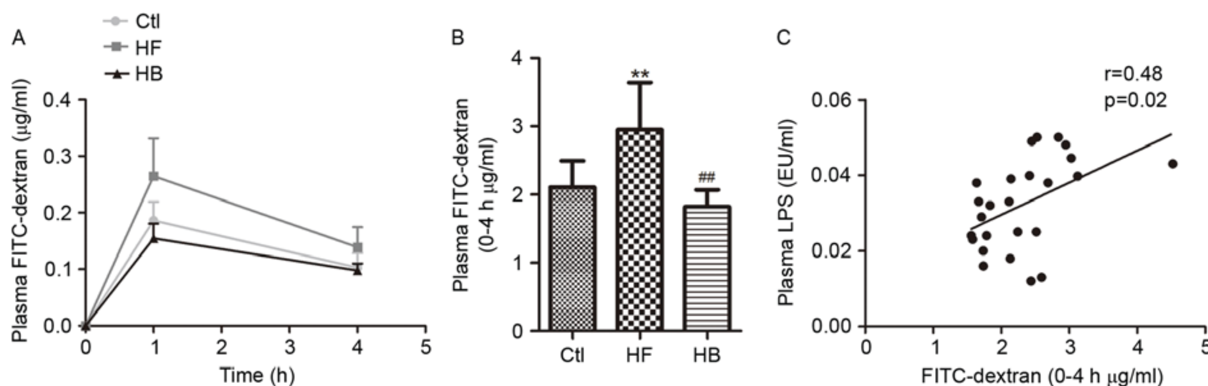

Figure 5. Berberine reduced intestinal permeability in HFD-fed rats. (A) Plasma FITC-dextran concentration. (B) Total plasma FITC-dextran concentration. (C) Correlation between portal plasma LPS levels and plasma FITC-dextran concentration. The inset corresponds to Pearson's correlation and the corresponding P-value. Data are expressed as the mean ± 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.

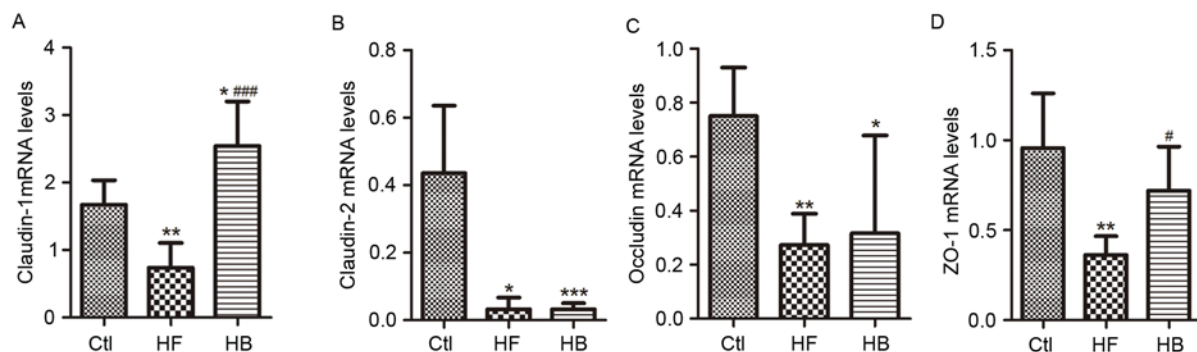

Figure 6. Berberine restored the expression of tight junction proteins in HFD-fed rats. mRNA expression levels of colonic epithelial tight junction proteins (A) claudin-1, (B) claudin-2, (C) occludin and (D) ZO-1. Data are expressed as the mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001 compared 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-1β, 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-1β, 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 TNFα, IL-1β, 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

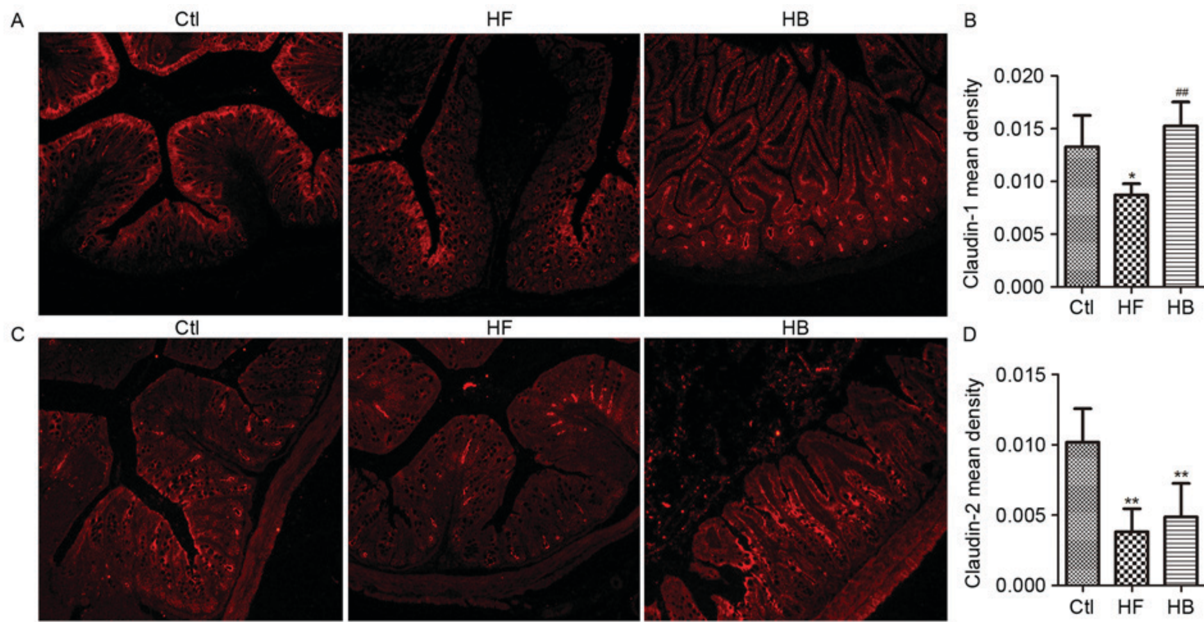

Figure 7. Berberine restored the expression and distribution of claudin-1 and claudin-2 in HFD-fed rats. (A) Immunofluorescent staining for claudin-1 (magnification, x100). (B) Immunohistochemistry score for claudin-1. (C) Immunofluorescent staining for claudin-2 (magnification, x100). (D) Immunohistochemistry score for claudin-2. Data are expressed as the mean ± standard deviation. *P<0.05, **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.

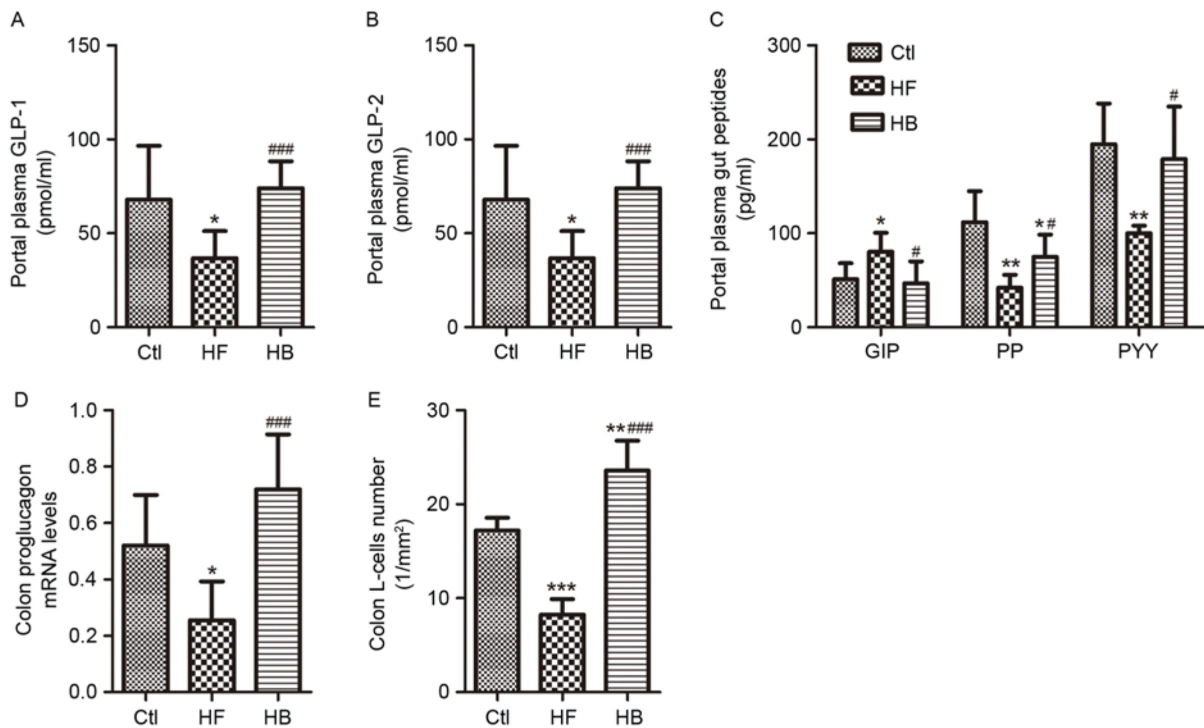

Figure 8. Berberine modulated intestinal hormone levels in portal plasma of HFD-fed rats. (A) Portal plasma GLP-1 levels. (B) Portal plasma GLP-2 levels. (C) Portal plasma GIP, PP and PYY levels. (D) Colonic proglucagon mRNA expression levels. (E) Colonic L-cell numbers. Data are expressed as the mean ± 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; 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

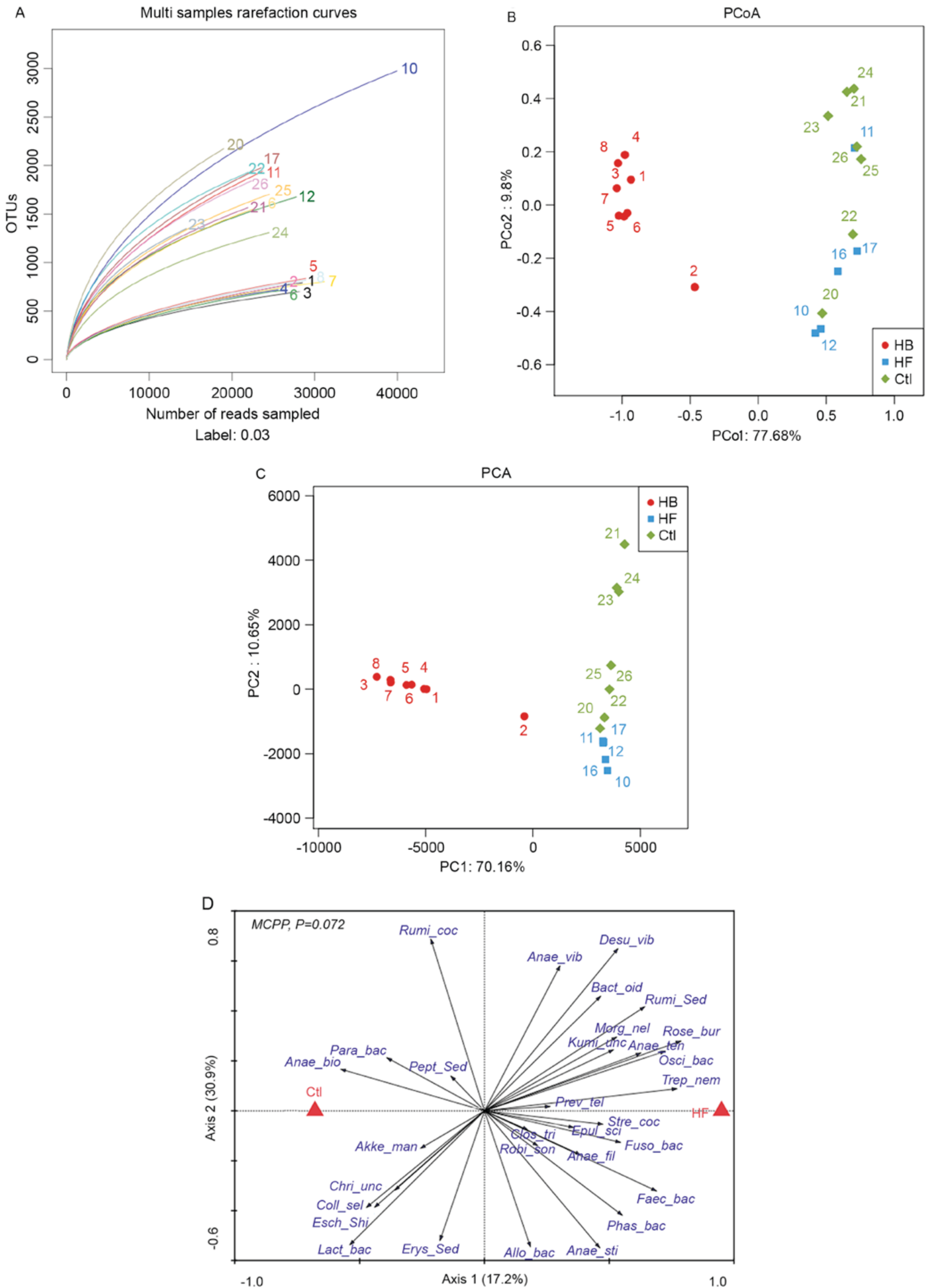

Figure 9. Berberine altered the gut microbiota composition in HFD-fed rats. (A) Rarefaction curves. (B) Weighted UniFrac PCoA. Each point represents the microbiota of each rat in the Ctl, HF and HB groups. (C) PCA. P-values were calculated by MCPP. (D) RDA between the Ctl and HF groups.

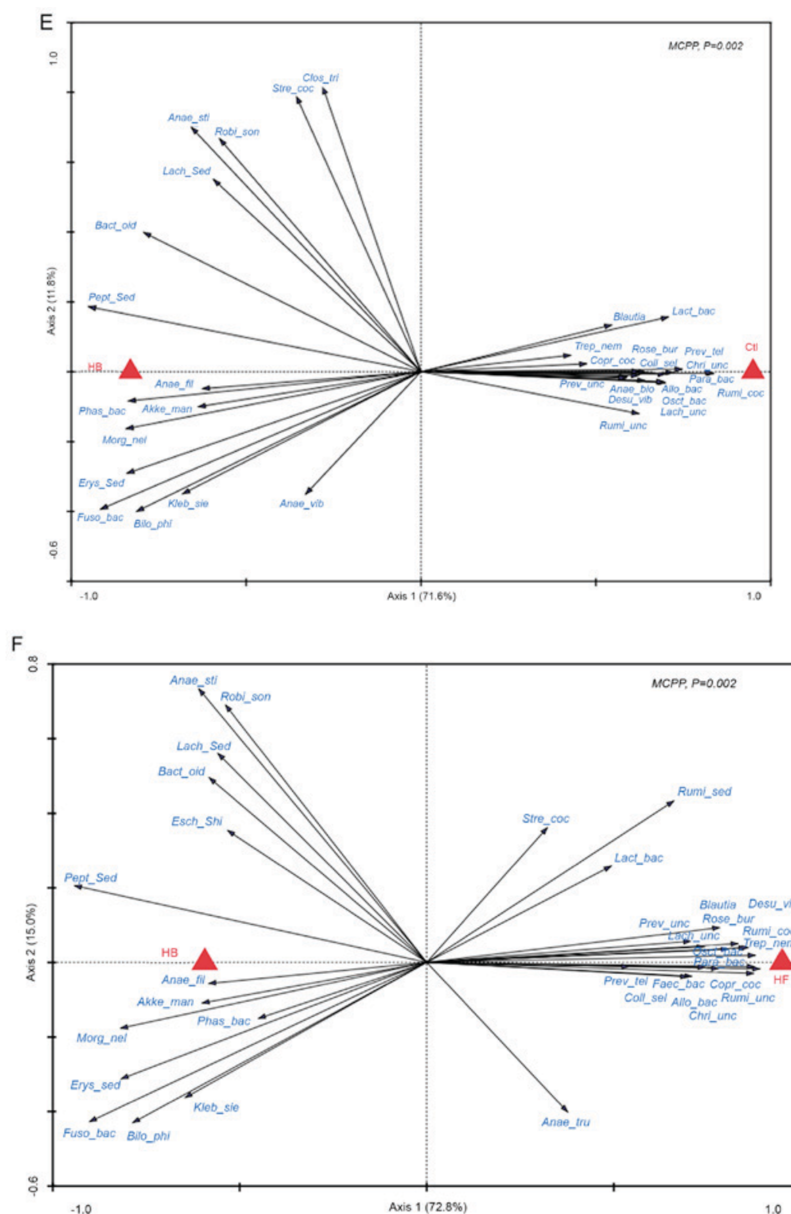

Figure 9. Continued. Berberine altered the gut microbiota composition in HFD-fed rats. (E) RDA between the Ctl and HB groups. (F) RDA between the HB and HF groups. P-values were calculated by MCPP. Ctl, normal diet; HF, HFD; HB, HFD supplemented with berberine for 6 weeks; HFD, high-fat diet; PCoA, principal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

[a]Sample names. [b]Trimmed 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 (MCPP; 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						
Acidobacteria	0.00135±0.00066	0.00293±0.0008	0.00199±0.00095	0.43870	0.71705	0.68879
Actinobacteria	0.02004±0.00564	1.94705±0.72971	7.24482±2.45339	0.01408	0.04507	0.00631
Bacteroidetes	11.37515±2.19301	14.64713±3.97861	7.62278±1.86003	0.51500	0.11850	0.18400
TM7	0.00182±0.0012	0.18116±0.09689	1.89118±0.6578	0.06685	0.01307	0.00685
Chloroflexi	0.00043±0.00043	0±0	0.00058±0.00058	1.00000	1.00000	1.00000
Cyanobacteria	0.00095±0.00095	0.08708±0.06498	0.06448±0.02119	0.20177	0.74114	0.00562
Deferribacteres	0±0	0.00314±0.00194	0.00756±0.00683	0.02102	0.57029	0.25377
Deinococcus-Thermus	0.00137±0.00095	0.00085±0.00085	0.00065±0.00065	1.00000	1.00000	1.00000
Elusimicrobia	0±0	0.00207±0.00089	0.00232±0.00114	0.05521	1.00000	0.06384
Firmicutes	34.07855±3.97821	79.44588±4.13566	79.69249±2.76575	0.00000	0.89414	0.00000
Fusobacteria	40.28194±4.39043	0.12066±0.05127	0.0163±0.00883	0.00000	0.05379	0.00000
Gemmatimonadetes	0.00095±0.00095	0±0	0.00058±0.00058	0.52842	1.00000	1.00000
Lentisphaerae	0±0	0.00968±0.00386	0.00389±0.00251	0.01669	0.22271	0.00408
Nitrospirae	0±0	0.00072±0.00072	0±0	0.38079	0.48016	1.00000
Planctomycetes	0.00044±0.00044	0.00157±0.00097	0±0	0.56199	0.23056	1.00000
Proteobacteria	11.05874±1.14416	2.45033±0.51587	2.29854±0.57357	0.00000	0.81821	0.00000
Spirochaetes	0.0013±0.0013	0.89612±0.16617	0.20734±0.11901	0.00015	0.00286	0.07762
Tenericutes	0.013±0.01151	0.12251±0.08587	0.88127±0.36557	0.22892	0.05143	0.02031
Verrucomicrobia	3.14836±1.01832	0.00072±0.00072	0.00646±0.00424	0.00469	0.19707	0.00492
Genus						
Acetanaerobacterium	0.00595±0.00473	0±0	0±0	0.22569	1.00000	0.22139
Acetobacteraceae_uncultured	0±0	0±0	0.00065±0.00065	1.00000	1.00000	0.39968
Acholeplasma	0±0	0±0	0.00117±0.00117	1.00000	0.50079	0.15974
Acidobacteriaceae_uncultured	0±0	0±0	0.00075±0.00075	1.00000	1.00000	0.39968
Acidothermus	0±0	0.00072±0.00072	0±0	0.38079	0.48016	1.00000
Acidovorax	0.00048±0.00048	0±0	0.00058±0.00058	1.00000	1.00000	1.00000
Acinetobacter	0.00179±0.00133	0±0	0±0	0.30479	1.00000	0.15540
Actinomyces	0.00315±0.00127	0.01949±0.01252	0.00564±0.00313	0.21147	0.30068	0.55334
Adlercreutzia	0±0	0.00562±0.00501	0.03181±0.00825	0.29136	0.01312	0.00060
Aeribacillus	0.00086±0.00086	0.00088±0.00088	0±0	1.00000	0.48016	0.52013
Aerococcaceae_uncultured	0.00044±0.00044	0±0	0±0	1.00000	1.00000	1.00000
Aerococcus	0±0	0.00088±0.00088	0±0	0.38079	0.48016	1.00000
Agrococcus	0.00043±0.00043	0±0	0±0	1.00000	1.00000	1.00000
Akkermansia	3.14836±1.01832	0±0	0.00447±0.00447	0.00472	0.34499	0.00353
Alcaligenes	0±0	0.00288±0.00288	0±0	0.02102	0.05316	1.00000
Alistipes	0±0	0.00423±0.00266	0.02031±0.00915	0.12302	0.10822	0.03099
Allobaculum	0.00311±0.00079	10.84206±3.94134	8.82073±2.9406	0.01252	0.74820	0.00424
Anaerobiospirillum	0.00049±0.00049	0.00088±0.00088	0.46779±0.17407	1.00000	0.01388	0.00936
Anaerofilum	2.30376±0.73263	0.05499±0.02737	0.0219±0.01234	0.00482	0.28611	0.00332
Anaerofustis	0±0	0.01313±0.01103	0.0104±0.00328	0.26065	0.84805	0.00274
Anaerolineaceae_uncultured	0±0	0±0	0.00058±0.00058	1.00000	1.00000	0.39968
Anaerostipes	1.29186±0.40432	0.04986±0.02226	0.01231±0.01159	0.00484	0.14894	0.00282
Anaerotruncus	0.77713±0.36482	1.53538±0.27758	0.81297±0.35685	0.10951	0.12560	0.94840
Anaerovibrio	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 ± SD	HF (%) mean ± SD	Ctl (%) mean ± SD	P-value		
				HB vs. HF	HF vs. Ctl	HB vs. Ctl
Anaerovorax	0±0	0.01789±0.00528	0.02409±0.00572	0.00299	0.51230	0.00026
Anoxybacillus	0.00183±0.00095	0.00072±0.00072	0.00239±0.00176	0.65574	0.37696	0.72123
Aquabacterium	0.00044±0.00044	0±0	0.0013±0.0013	1.00000	0.50079	0.56788
Arenimonas	0±0	0±0	0.00065±0.00065	1.00000	1.00000	0.39968
Bacillus	0.00438±0.00295	0±0	0.00129±0.00083	0.14862	0.50079	0.33974
Bacteroides	11.32657±2.19329	2.86663±1.50674	0.50319±0.25937	0.00396	0.13701	0.00010
Barnesiella	0±0	0±0	0.00064±0.00064	1.00000	1.00000	0.39968
Bifidobacterium	0±0	0.00085±0.00085	0±0	0.38079	0.48016	1.00000
Bilophila	0.79589±0.15162	0.00085±0.00085	0.00157±0.00106	0.00046	1.00000	0.00003
Blautia	0.80417±0.24782	6.23756±1.76412	6.90259±2.62767	0.00509	0.86245	0.02394
Brachybacterium	0±0	0±0	0.00197±0.00197	1.00000	0.50079	0.15974
Bradyrhizobium	0±0	0±0	0.00065±0.00065	1.00000	1.00000	0.39968
Brevundimonas	0.00216±0.00113	0±0	0±0	0.16413	1.00000	0.16476
Burkholderia	0.01374±0.00306	0.00895±0.00321	0.00898±0.00396	0.31982	0.98550	0.42879
Butyricicoccus	0±0	0±0	0.00058±0.00058	1.00000	1.00000	0.39968
Butyricimonas	0±0	0.00602±0.00423	0.00075±0.00075	0.16249	0.23333	0.39968
Candidatus_ Arthromitus	0±0	0.0005±0.0005	0.00559±0.00411	0.38079	0.23120	0.18080
Candidatus_ Chloracidobacterium	0.00091±0.00059	0.00135±0.00087	0±0	0.63837	0.23056	0.52013
Candidatus_ Solibacter	0±0	0±0	0.00065±0.00065	1.00000	1.00000	0.39968
Caulobacter	0.00044±0.00044	0±0	0±0	1.00000	1.00000	1.00000
Cellulosilyticum	0.00048±0.00048	0±0	0±0	1.00000	1.00000	1.00000
Christensenellaceae_ uncultured	0±0	0.50197±0.122	1.05035±0.29907	0.00139	0.10517	0.00119
Christensenella	0±0	0.00765±0.00241	0.07967±0.01597	0.00398	0.00051	0.00007
Chryseobacterium	0.00136±0.00066	0±0	0±0	0.29263	1.00000	0.28019
Clostridium	1.0162±0.90173	0.00465±0.00191	0.0035±0.00288	0.29069	0.79322	0.27462
Collinsella	0±0	1.75193±0.70971	6.94762±2.43052	0.02134	0.05349	0.00609
Comamonas	0.00588±0.00213	0.00229±0.00148	0.00369±0.00194	0.18018	0.51090	0.54084
Coprococcus	0.00187±0.00101	0.34846±0.07673	0.44409±0.23277	0.00092	0.76015	0.06288
Coriobacteriaceae_ uncultured	0.00044±0.00044	0.07323±0.01542	0.07895±0.02065	0.00080	0.85713	0.00067
Corynebacterium	0±0	0.00751±0.00291	0.00305±0.00119	0.01717	0.17098	0.01020
Deinococcus	0.00137±0.00095	0.00085±0.00085	0.00065±0.00065	1.00000	1.00000	1.00000
Delftia	0.02537±0.00587	0.00866±0.00411	0.01068±0.00254	0.02783	0.74368	0.02491
Desemzia	0.00048±0.00048	0±0	0±0	1.00000	1.00000	1.00000
Desulfovibrio	0.13322±0.0136	2.12679±0.44718	1.30345±0.47798	0.00099	0.22106	0.01686
Devosia	0.00092±0.0006	0±0	0±0	0.52842	1.00000	0.52013
Diaphorobacter	0.00049±0.00049	0±0	0.00117±0.00117	1.00000	0.50079	0.56788
Elusimicrobium	0±0	0.00207±0.00089	0.00232±0.00114	0.05521	1.00000	0.06384
Enhydrobacter	0.00084±0.00055	0±0	0±0	0.52842	1.00000	0.52013
Enterococcus	0.3229±0.06599	0.002±0.002	0.00649±0.00376	0.00075	0.31480	0.00011
Enterorhabdus	0.00043±0.00043	0.01485±0.00579	0.07087±0.0206	0.02093	0.01589	0.00164
Epulopiscium	0.1753±0.17421	0.00088±0.00088	0±0	0.36952	0.48016	0.34371
Erysipelothrix	0.00096±0.00096	0±0	0±0	0.52842	1.00000	0.52013
Erysipelotrichaceae_ Incertae_Sedis	2.50531±0.39665	0.00672±0.00292	0.01301±0.00902	0.00017	0.58704	0.00002

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
Erysipelotrichaceae_ uncultured	0±0	0.00648±0.00587	0.01533±0.00785	0.30366	0.45472	0.05456
Escherichia-Shigella	0.35447±0.11707	0.02743±0.00973	0.32604±0.1537	0.01092	0.07005	0.90572
Faecalibacterium	0.00173±0.00131	0.81438±0.32885	0.00058±0.00058	0.02116	0.02256	0.65440
Family_XIII_ Incertae_Sedis_ Incertae_Sedis	0±0	0.10057±0.02552	0.20788±0.04714	0.00163	0.06129	0.00018
Family_XIII_ Incertae_Sedis_ uncultured	0±0	0.36272±0.09627	0.21403±0.05279	0.00193	0.19001	0.00036
Ferruginibacter	0.0008±0.0008	0±0	0.00058±0.00058	0.52842	1.00000	1.00000
Flavobacterium	0.00096±0.00096	0.00264±0.00264	0.00058±0.00058	0.37584	0.35637	1.00000
Flavonifractor	0.01386±0.01386	0.00451±0.00451	0.0693±0.05199	0.61083	0.22530	0.32534
Flexibacter	0.00048±0.00048	0±0	0.0013±0.0013	1.00000	0.50079	0.56788
Fusobacterium	40.17087±4.4684	0.03271±0.01065	0.01066±0.00678	0.00004	0.09584	0.00000
GKS98_freshwater_ group	0.00088±0.00088	0±0	0±0	0.52842	1.00000	0.52013
Gelria	0±0	0.0005±0.0005	0±0	0.38079	0.48016	1.00000
Gemella	0.00776±0.00299	0.16081±0.06141	0.00508±0.00187	0.02078	0.01988	0.53684
Gemmatimonadac eae_uncultured	0.00095±0.00095	0±0	0±0	0.52842	1.00000	0.52013
Geobacillus	0.00087±0.00057	0±0	0±0	0.52842	1.00000	0.52013
Globicatella	0±0	0±0	0.00129±0.00083	1.00000	0.50079	0.15974
Granulicatella	0±0	0.00088±0.00088	0±0	0.38079	0.48016	1.00000
Haliangium	0.00048±0.00048	0±0	0±0	1.00000	1.00000	1.00000
Helicobacter	0.07094±0.02612	0.03422±0.01028	0.01257±0.006	0.20991	0.08585	0.03429
Herbaspirillum	0±0	0.00144±0.00144	0±0	0.14500	0.23056	1.00000
Holdemania	0±0	0.06632±0.01785	0.09885±0.0582	0.00208	0.66766	0.09437
Hydrogenoanaero *bacterium*	0±0	0.0005±0.0005	0±0	0.38079	0.48016	1.00000
Hydrogenophaga	0±0	0.00072±0.00072	0.00065±0.00065	0.38079	1.00000	0.39968
Iamia	0.00048±0.00048	0±0	0±0	1.00000	1.00000	1.00000
Janthinobacterium	0.0004±0.0004	0±0	0±0	1.00000	1.00000	1.00000
Jeotgalicoccus	0.00044±0.00044	0±0	0±0	1.00000	1.00000	1.00000
Klebsiella	2.78132±0.76059	0.0005±0.0005	0.00122±0.00079	0.00220	1.00000	0.00088
Kocuria	0.00654±0.00483	0±0	0±0	0.19016	1.00000	0.18444
Lachnospiraceae_ Incertae_Sedis	3.56313±0.69197	1.58603±0.57302	1.85636±0.36867	0.03981	0.75605	0.03432
Lachnospiraceae_ uncultured	0.01129±0.00569	6.64096±2.13871	10.66917±3.4409	0.00469	0.39685	0.00338
Lachnospira	0±0	0.00171±0.00171	0.0047±0.00389	0.14500	0.45570	0.01020
Lactobacillus	0.69316±0.42232	3.77028±2.08403	16.53313±5.33947	0.15694	0.03801	0.00458
Lactococcus	0.0058±0.00206	0.00072±0.00072	0±0	0.02900	0.48016	0.00690
Leifsonia	0±0	0±0	0.00065±0.00065	1.00000	1.00000	0.39968
Leptolyngbya	0±0	0±0	0.00254±0.00194	1.00000	0.12618	0.02552
Leptothrix	0.00043±0.00043	0±0	0±0	1.00000	1.00000	1.00000
Leucobacter	0.00044±0.00044	0.00088±0.00088	0±0	1.00000	0.48016	1.00000
Leuconostoc	0.00314±0.00103	0±0	0±0	0.04928	1.00000	0.04686
Luteimonas	0±0	0±0	0.00099±0.00099	1.00000	1.00000	0.39968
Lysobacter	0±0	0±0	0.00058±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
Marmoricola	0.00048±0.00048	0±0	0±0	1.00000	1.00000	1.00000
Marvinbryantia	0±0	0.15705±0.04201	0.13174±0.03237	0.00203	0.70828	0.00033
Megamonas	0±0	0±0	0.00058±0.00058	1.00000	1.00000	0.39968
Methylobacillus	0±0	0±0	0.00065±0.00065	1.00000	1.00000	0.39968
Methylobacterium	0.00084±0.00055	0±0	0.00117±0.00117	0.52842	0.50079	0.65412
Microbacterium	0±0	0.00176±0.00176	0±0	0.14500	0.23056	1.00000
Micrococcus	0.0004±0.0004	0±0	0±0	1.00000	1.00000	1.00000
Mogibacterium	0±0	0.10606±0.02317	0.06192±0.03252	0.00090	0.28500	0.06173
Morganella	0.63669±0.10501	0.00122±0.00077	0±0	0.00024	0.23056	0.00002
Mucilaginibacter	0±0	0±0	0.0013±0.0013	1.00000	0.50079	0.15974
Mucispirillum	0±0	0.00314±0.00194	0.00756±0.00683	0.02102	0.61466	0.28153
Mycobacterium	0.00088±0.00058	0±0	0±0	0.52842	1.00000	0.52013
Mycoplasma	0.013±0.01151	0±0	0.00075±0.00075	0.28651	1.00000	0.30851
Nesterenkonia	0±0	0±0	0.00064±0.00064	1.00000	1.00000	0.39968
Nitrospiraceae_ uncultured	0±0	0.00072±0.00072	0±0	0.38079	0.48016	1.00000
Ochrobactrum	0.01515±0.00334	0.00684±0.00496	0.00507±0.00217	0.17780	0.79573	0.01367
Odoribacter	0.02862±0.01189	0.0005±0.0005	0.00304±0.00111	0.02645	0.37696	0.03689
Opitutus	0±0	0.00072±0.00072	0.00198±0.00094	0.38079	0.62618	0.06384
Oscillibacter	0.00134±0.00092	1.91854±0.4873	0.47583±0.15936	0.00169	0.01063	0.00438
Oscillospira	0.00043±0.00043	0.1396±0.06182	0.05487±0.0542	0.03471	0.32838	0.34728
Ottowia	0±0	0±0	0.00409±0.00409	1.00000	0.34499	0.35432
Oxalobacter	0±0	0.04147±0.01443	0±0	0.00911	0.00915	1.00000
Paenisporosarcina	0±0	0±0	0.00233±0.00233	1.00000	0.12618	0.02552
Papillibacter	0±0	0.00885±0.00248	0.01761±0.00523	0.00235	0.14454	0.00181
Parabacteroides	0.00087±0.00057	0.29747±0.10585	0.76933±0.24052	0.01072	0.08878	0.00256
Pasteurella	0.0104±0.00628	0.00144±0.00144	0.00442±0.00327	0.17627	0.49073	0.49161
Paucimonas	0±0	0.00072±0.00072	0±0	0.38079	0.48016	1.00000
Pelomonas	0.00266±0.00114	0±0	0.00058±0.00058	0.08916	1.00000	0.25499
Peptococcaceae_ uncultured	0±0	0.11683±0.04023	0.05492±0.01607	0.00863	0.16894	0.00164
Peptococcus	0±0	0.01105±0.00382	0.01071±0.00654	0.00879	0.96040	0.10656
Peptostreptococc aceae_Incertae_Sedis	9.05387±0.70459	0.05952±0.03087	0.11667±0.03346	0.00000	0.22173	0.00000
Peptostreptococcus	0.00048±0.00048	0±0	0±0	1.00000	1.00000	1.00000
Phascolarcto bacterium	4.99382±0.69833	3.28502±1.54339	0.532±0.40397	0.36342	0.09938	0.00003
Phyllobacterium	0.00188±0.00101	0±0	0±0	0.30479	1.00000	0.15540
Pir4_lineage	0.00044±0.00044	0±0	0±0	1.00000	1.00000	1.00000
Prevotellaceae_ uncultured	0±0	0.64183±0.16969	0.9912±0.41729	0.00190	0.52234	0.02007
Prevotella	0.00545±0.00269	1.28652±0.75418	0.93156±0.2499	0.10279	0.72772	0.00083
Propionibacterium	0.00362±0.00157	0.004±0.00177	0.00122±0.00079	0.89485	0.16840	0.17603
Proteiniphilum	0±0	0±0	0.00467±0.00467	1.00000	0.34499	0.35432
Proteus	0.03882±0.00637	0±0	0±0	0.00021	1.00000	0.00002
Pseudomonas	0±0	0±0	0.00376±0.00376	1.00000	0.06348	0.01020
Pseudorhodoferax	0.0008±0.0008	0±0	0±0	0.52842	1.00000	0.52013
Pseudoxanthomonas	0.00048±0.00048	0.002±0.002	0±0	0.07310	0.05316	1.00000
RC9_gut_group	0±0	0.2138±0.0391	0.25933±0.08393	0.00037	0.69826	0.00352
Ramlibacter	0±0	0.00216±0.00216	0±0	0.05521	0.11070	1.00000

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

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
vadinBC27_waste water-sludge_group	0±0	0±0	0.00075±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-1β 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 (%)
Akkermansia	Akke_man	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	3.14836±1.01832	0.00000±0.00000	HB[a]
Anaerofilum	Anae_fil	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	2.30376±0.73263	0.05499±0.02737	4089.2
Anaerostipes	Anae_sti	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	1.29186±0.40432	0.04986±0.02226	2490.8
Bacteroides	Bact_oid	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	11.32657±2.19329	2.86663±1.50674	295.1
Bilophila	Bilo_phi	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	0.79589±0.15162	0.00085±0.00085	93122.1
Erysipelotrichaceae _Incertae_Sedis	Erys_Sed	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	2.50531±0.39665	0.00672±0.00292	37190.7
Escherichia-Shigella	Esch_Shi	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	0.35447±0.11707	0.02743±0.00973	1192.4
Fusobacterium	Fuso_bac	Fusobacteria	Fusobacteria	Fusobacteriales	Fusobacteriaceae	40.17087±4.46840	0.03271±0.01065	122701.9
Klebsiella	Kleb_sie	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	2.78132±0.76059	0.00050±0.00050	555400.0
Lachnospiraceae_ Incertae_Sedis	Lach_Sed	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	3.56313±0.69197	1.58603±0.57302	124.7
Morganella	Morg_nel	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	0.63669±0.10501	0.00122±0.00077	52081.4
Peptostreptococcaceae_ Incertae_Sedis	Pept_Sed	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	9.05387±0.70459	0.05952±0.03087	15110.5
Phascolarctobacterium	Phas_bac	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	4.99382±0.69833	3.28502±1.54339	52.0
Robinsoniella	Robi_son	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	1.60965±0.60768	0.00171±0.00171	94285.6
Allobaculum	Allo_bac	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	0.00311±0.00079	10.84206±3.94134	-100.0
Anaerotruncus	Anae_tru	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.77713±0.36482	1.53538±0.27758	-49.4
Blautia	Blautia	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	0.80417±0.24782	6.23756±1.76412	-87.1
Christensenellaceae_ uncultured	Chri_unc	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	0.00000±0.00000	0.50197±0.12200	HF[b]
Collinsella	Coll_sel	Actinobacteria	Actinobacteria	Coriobacteriales	Coriobacteriaceae	0.00000±0.00000	1.75193±0.70971	HF[b]
Coprococcus	Copr_coc	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	0.00187±0.00101	0.34846±0.07673	-99.5
Desulfovibrio	Desu_vib	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	0.13322±0.01360	2.12679±0.44718	-93.7
Faecalibacterium	Faec_bac	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.00173±0.00131	0.81438±0.32885	-99.8
Lachnospiraceae_ uncultured	Lach_unc	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	0.01129±0.00569	6.64096±2.13871	-99.8
Lactobacillus	Lact_bac	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	0.69316±0.42232	3.77028±2.08403	-81.6
Oscillibacter	Osci_bac	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.00134±0.00092	1.91854±0.48730	-99.9
Parabacteroides	Para_bac	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	0.00087±0.00057	0.29747±0.10585	-99.7
Prevotella	Prev_tel	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	0.00545±0.00269	1.28652±0.75418	-99.6
Prevotellaceae_ uncultured	Prev_unc	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	0.00000±0.00000	0.64183±0.16969	HF[b]

Table IV. Continued.

Genus	Genus for short	Phylum	Class	Order	Family	HB (%)	HF (%)	Change (%)
Roseburia	Rose_bur	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	0.00043±0.00043	3.29178±0.50767	-100.0
Ruminococcaceae_ Incertae_Sedis	Rumi_Sed	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.53333±0.18071	1.43560±0.21452	-62.8
Ruminococcaceae_ uncultured	Rumi_unc	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	1.58302±0.31620	13.47235±2.72614	-88.2
Ruminococcus	Rumi_coc	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	0.00000±0.00000	0.40738±0.06601	HF[b]
Streptococcus	Stre_coc	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	0.34317±0.15446	0.85093±0.48484	-59.7
Treponema	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: [a]HB, found only in berberine-treated rats; [b]HF, 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-IR	AUC of OGTT	FITC-dextran area	claudin -1	claudin -2	ZO -1	occludin	L-cells	GLP -1	GLP -2	GIP	PP	PYY
Akkermansia	-0.467	ns	ns	ns	ns	ns	ns	ns	ns	0.558	0.628	ns	ns	ns	ns	ns
Anaerofilum	ns	ns	ns	ns	0.537	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Anaerostipes	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.56	ns	ns	ns	ns	ns
Bacteroides	ns	ns	ns	ns	ns	ns	0.436	ns	ns	ns	ns	0.54	ns	ns	ns	ns
Bilophila	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.558	ns	ns	ns	ns	ns
Escherichia-Shigella	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fusobacterium	ns	ns	ns	ns	ns	ns	0.595	ns	ns	ns	ns	0.796	0.524	ns	ns	ns
Klebsiella	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Lachnospiraceae_ Incertae_Sedis	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Morganella	ns	ns	ns	ns	ns	ns	ns	0.582	0.507	0.495	ns	ns	ns	ns	ns	ns
Peptostreptococcaceae _Incertae_Sedis	-0.462	ns	ns	ns	ns	ns	0.524	ns	ns	ns	ns	ns	ns	ns	ns	ns
Phascolarctobacterium	ns	ns	ns	ns	ns	-0.522	ns	ns	0.483	ns	ns	ns	ns	ns	ns	ns
Robinsoniella	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.683	ns	ns
Allobaculum	ns	-0.531	ns	ns	ns	ns	ns	-0.531	ns	ns	ns	ns	-0.643	ns	ns	ns
Anaerotruncus	0.683	ns	ns	ns	ns	0.435	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Blautia	ns	-0.605	ns	ns	ns	ns	ns	-0.474	ns	ns	ns	-0.52	-0.867	ns	ns	-0.496
Christensenellaceae _uncultured	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Collinsella	ns	ns	ns	ns	ns	-0.792	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Coprococcus	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Desulfovibrio	ns	ns	ns	ns	ns	ns	-0.521	ns	ns	ns	ns	ns	ns	ns	ns	ns
Faecalibacterium	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Lachnospiraceae _uncultured	ns	ns	ns	ns	ns	ns	-0.524	ns	ns	ns	ns	ns	ns	ns	ns	ns
Lactobacillus	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.541	-0.503	ns	ns	ns	ns
Oscillibacter	ns	0.525	0.565	0.543	0.697	0.473	ns	ns	-0.638	ns	0.522	ns	ns	ns	-0.721	ns
Parabacteroides	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.817
Prevotella	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Prevotellaceae _uncultured	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Roseburia	0.555	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.617	-0.738
Ruminococcaceae_ Incertae_Sedis	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

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
Ruminococcaceae _uncultured	0.441	ns	ns	ns	ns	ns	-0.541	ns	ns	ns	ns	-0.552	ns	ns	ns	ns
Ruminococcus	ns	ns	ns	ns	ns	ns	ns	0.602	ns	ns	ns	ns	ns	ns	ns	ns
Streptococcus	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.514
Treponema	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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