Thieme

Berberine Modulates Gut Microbiota and Reduces Insulin Resistance via the TLR4 Signaling Pathway

Authors
Dan Liu[1*], Yiyi Zhang[1*], Yunhui Liu[2], Liqiong Hou[3], Sheyu Li[1], Haoming Tian[1], Tieyun Zhao[1]

Affiliations
1 Department of Endocrinology and Metabolism, West China Hospital, Sichuan University, Chengdu 610041, China
2 Department of Endocrinology, Liuzhou Worker's Hospital, Liuzhou 545005, China
3 Department of Rheumatology, the First Hospital of Lanzhou University, Lanzhou 730000, China

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Correspondence
Tieyun Zhao
Department of Endocrinology and Metabolism
West China Hospital
Sichuan University
GuoXue Lane 37
Chengdu
Sichuan, 610041
China
Tel.: +86/189/8060 1308, Fax: +86/28/8542 2982
zty112356@gmail.com

ABSTRACT

Berberine, a natural compound extracted from several Chinese herbs including Coptis chinensis, has been shown to have anti-obesity effects and prevents insulin resistance in high-fat diet (HFD)-fed obese rats by modulating the gut microbiota; however, the molecular mechanisms underlying these activities remain unknown. We investigated the effects of berberine on obesity and insulin resistance by examining the lipopolysaccharide (LPS)/toll-like receptor 4 (TLR4)/tumor necrosis factor (TNF)-α signaling pathway in livers of HFD-fed obese rats. Our results showed that 8-week berberine (200 mg/kg) treatment significantly reduced fasting blood glucose, triglyceride, low-density lipoprotein-cholesterol and insulin resistance in HFD-fed obese rats. However, berberine had no significant effects on body weight, visceral fat mass or the visceral fat to body weight ratio. Berberine also attenuated HFD-induced hepatic steatosis. A prolonged HFD altered the gut microbiota composition by reducing protective bacteria like Bifidobacterium and increasing gram negative bacteria like Escherichia coli, which resulted in increased LPS release into plasma. Berberine reversed these effects and inhibited LPS-induced TLR4/TNF-α activation, resulting in increased insulin receptor and insulin receptor substrate-1 expression in the liver. These findings suggested that berberine may reduce insulin resistance, at least in part by modulating the gut microbiota along with inhibiting LPS/TLR4/TNF-α signaling in the liver.

Introduction

Worldwide, the prevalence of obesity and obesity-related metabolic disorders including insulin resistance, type 2 diabetes mellitus (T2DM) and cardiovascular disease is increasing rapidly, imposing substantial health and economic burdens on society [1]. Obesity is a complex disease with both genetic and environmental causes. Accumulating evidence suggests that obesity is associated with a low-grade systemic and chronic inflammatory condition that induces insulin resistance [2].

In animal models, a high-fat diet (HFD) leads to gut microbiota dysbiosis and increased intestinal permeability, thus allowing translocation of microbiome-derived lipopolysaccharides (LPS) (also known as endotoxin) into the bloodstream [3]. This causes a 2-3-fold increase in plasma LPS concentration, a threshold defined as metabolic endotoxemia [3]. Delivered by LPS-binding protein (LBP) in the serum, LPS binds to the complex of CD14 and toll-like receptor 4 (TLR4) on the surface of innate immune cells, leading to the

[*] These authors contributed equally to this work.

activation of multiple inflammatory signaling pathways and the expression of inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6 [4, 5]. Proinflammatory cytokines deleteriously influence insulin signaling. For example, TNF-α has been shown to induce insulin resistance by increasing the phosphorylation of inactivating serine residues on insulin receptor substrate (IRS)-1, and reducing insulin receptor (IRc) and IRS-1 expression [6, 7]. It has been demonstrated that metabolic endotoxemia along with LPS/CD14 signaling controls inflammation and triggers HFD-induced insulin resistance and obesity [3]. Under such circumstances, the gut microbiota, known as our second genome, has increasingly been recognized as a key factor linking genetics, environment and the immune system to the development of obesity [8]. In the past few years, modulating gut microbiota has been shown to be a new therapeutic approach for treating obesity and obesity-related metabolic disorders [9–11].

Berberine, a natural plant alkaloid extracted from several Chinese herbs such as Coptis chinensis (Huanglian), has long been used to treat diarrhea in China [12]. Low-dose berberine has also been proven to have beneficial effects for T2DM and dyslipidemia with few side effects [13, 14]. Additionally, previous studies have shown that berberine may have anti-obesity effects and reduce insulin resistance in obese rodent models by modulating the gut microbiota and reducing intestinal endotoxins (LPS) in the bloodstream [15, 16]. A previous study reported that the liver is the first target of LPS-induced insulin resistance [3], but is still unknown whether berberine alleviates insulin resistance and prevents obesity by modulating endotoxin-related signaling pathways in the liver.

In this study, we tested the hypothesis that the protective effects of berberine on HFD-induced obesity and insulin resistance in rats involve modulating the gut microbiota and the LPS/TLR4/TNF-α signaling pathway in the liver.

Materials and Methods

Materials

A total of forty specific pathogen free (SPF) male Wistar rats (aged 6 weeks, weight 210 ± 10 g) were purchased from Dashuo Laboratory Animal Co., Ltd (Chengdu, Sichuan, China) and housed at the SPF animal facility of West China Hospital of Sichuan University (temperature: 22 ± 2 °C; 12 h light/dark cycle) with free access to food and water. Both high-fat diet (HFD, total energy: carbohydrates = 34 %, fats = 58 %, and proteins = 8 %) and normal chow diet (NCD, total energy: carbohydrates = 68 %, fats = 18 %, and proteins = 14 %) for rats were purchased from Dashuo Laboratory Animal Co., Ltd (Chengdu, Sichuan, China). Berberine hydrochloride (99.5 %) was extracted from the dry root of Coptidis chinensis and purchased from Hongyi Bio-Engineering Co., Ltd (Chengdu, Sichuan, China). Berberine solutions were prepared in sterilized distilled water and given to rats by gavage. Non-treatment group was gavaged daily with 2.4 mL/kg sterilized distilled water to minimize the effects of gavage procedure.

After one week's acclimatization, all the forty rats were randomly divided into 2 groups: (1) normal control (NC) group (n = 10), fed with NCD diet; (2) HFD group (n = 30), raised with HFD diet. After 28 weeks, rats feeding with HFD attained significantly higher average body weight (BW) (20 %) relative to the NC group, and then the obese rat model was considered to be successfully established [17]. After the successful establishment of an obese rat model, the NC group rats (n = 10) and 10 randomly selected HFD group obese rats (HF1 group) were sacrificed. And the remaining 20 HFD-fed obese rats were randomly assigned into 2 groups: (1) HF2 group (n = 10), continually fed with HFD and gavaged daily with sterilized distilled water for eight weeks; (2) HF + BBR group (n = 10), fed with HFD and gavaged daily with berberine at a dose with 200 mg/kg for eight weeks. The dose of berberine chosen for current study was based on a previous study, which has reported an optimal glucose lowering effect with 200 mg/kg of berberine [18]. Throughout the experimental period, BW was monitored once weekly and dose of berberine was adjusted according to BW. Fresh fecal samples of all rats were collected a day before sacrifice into sterile tubes and stored at -20 °C until analysis. At the end of 28th and 36th week, after 12 h fasting, fasting BW of each rat was assessed and rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). Blood was drawn from portal vein. Fasting blood glucose (FBG) concentrations were immediately measured by a glucose meter. Serum and plasma were separated from the remaining blood and stored at -80 °C. Visceral fat mass (VFM) and liver were dissected and weighed after being dried on filter papers. The visceral fat (VF) to BW ratio (VF/BW) was calculated. A small fragment of liver was fixed in 4 % formaldehyde, embedded in paraffin and then processed to Hematoxylin and Eosin (H&E) staining. A small fragment of intestine (approximately 1 cm proximal to the ileocecal junction) was dissected, flushed off the contents and prepared for H&E staining as said above. The remaining liver samples were snap-frozen in liquid nitrogen and then stored at -80 °C. Animals used in this study were maintained in accordance with the Guide for Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication No. 85-23, revised 1996) and the Policy of Animal Care and Use Committee of Sichuan University.

Biochemical analyses

The plasma endotoxin concentrations were assayed by the endpoint chromogenic limulus amebocyte using a commercially available kit purchased from Xiamen Houshiji Ltd. (Xiamen, China). Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) concentrations were determined by using commercially available kits manufactured by Changchun Huili Biotech Co., Ltd. (Changchun, China). Fasting serum insulin levels were measured by a ELISA kit from EMD Millipore (Billerica, MA, USA). The Homeostasis Model Assessment of Insulin Resistance (HOMA1-IR) index was calculated using fasting plasma insulin and glucose by the following formula:

HOMA1-IR = fasting plasma insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5.

Histology

Liver and intestinal tissues embedded in paraffin were cut into 5-μm thick consecutive sections, and were stained with H&E for evaluation of cellular morphological changes. The slides were observed with a phase-contrast microscope (Olympus IX70; Olympus Cor-

▶ **Table 1** Primers used in this study.

Target organism	Primer sequence 5'-3'	PCR Product (bp)	References
All bacteria	Fwd. CGGCAACGAGCGCAACCC Rev. CCATTGTAGCACGTGTGTAGCC	146	Bahl et al., 2012
Enterococcus spp.	Fwd. CCCTTATTGTTAGTTGCCATCATT Rev. ACTCGTTGTACTTCCCATTGT	144	Bahl et al., 2012
Lactobacillus	Fwd. GCAGCAGTAGGGAATCTTCCA Rev. GCATTYCACCGCTACACATG	340	Verma et al., 2012
Bifidobacterium	Fwd. GATTCTGGCTCAGGATGAACGC Rev. CTGATAGGACGCGACCCCAT	231	Verma et al., 2012
Escherichia coli	Fwd. CATGCCGCGTGTATGAAGAA Rev. CGGGTAACGTCAATGAGCAAA	96	Penders et al., 2007

poration, Tokyo, Japan). Lobular inflammation was graded on a four-point scale: 0 (no foci), 1 (< 2 foci per 200 × field), 2 (2-4 foci per 200 × field), or 3 (> 4 foci per 200 × field).

RNA isolation and gene expression analysis

Total RNA was isolated from the liver using the E.Z.N.A.TM Total RNA Kit II (Omega, USA) according to the manufacturer's instructions. Quality and concentration of RNA were checked using a NanoDrop 2000c Spectrophotometer (Thermo Scientific). 1 μg of RNA was reverse-transcribed with random hexamers using SuperScript II Reverse Transcriptase System (Invitrogen). Real-time quantitative PCR (qPCR) was performed with FastStart Universal SYBR Green Master (Rox) in a CFX96 real-time PCR detection system (Bio-Rad, USA) and each reaction was carried out in triplicate. Relative mRNA expression levels of candidate genes were calculated by the comparative CT method and all data were normalized to β-actin mRNA levels. The following primers were used: 5′-GCCGG AAAGTTATT-GTGGTGGT-3′ and 5′-ATGGGTTTAG GCGCAAAGT TT-3′ for TLR4; 5′-TGGCCCAGACCCTCACACTC -3′ and 5′-CTCCTGGTATGAAAT GGCAAATC-3′ for TNF-α; 5′-GTGCAAACA GATGCCACCAA-3′ and 5′-TCAGCAC CATTGCCTGAAGA-3′ for IRc; 5′-ACCCACTCCT ATCCC G-3′ and 5′-CCCTACTCC GTTTGTCC-3′ for IRS-1; 5′-CTATCGGCAAT-GAGCGGTTCC -3′ and 5′-T GTGTTGG CATAGAGGTCTTTACG-3′ for β-actin (control).

Western blot analysis

Frozen liver tissues were homogenized in RIPA buffer, and total protein was harvested and quantitated by standard Bradford assays. Western blotting was carried out by standard procedures and probed with primary antibody against TLR4 (1:1000; Cell Signaling, USA) or TNF-α (1:1000; Cell Signaling, USA) followed by the peroxidase-conjugated secondary antibody (1:5000; ZSGB-BIO, Beijing, China). Protein expression was visualized by the Molecular Imager ChemiDoc XRS + System (Bio-Rad, USA) and quantified by Odyssey software (LI-COR Biosciences, Lincoln, NE). Results were normalized relative to β-actin (1:1000; ZSGB-BIO, Beijing, China) expression for variation in loading and transfer.

Bacterial genomic DNA extraction

Bacterial genomic DNA was extracted from fecal samples using a TIANamp stool DNA kit (Tiangen Biotech Co. Ltd., Beijing, China) according to the manufacturer's instructions. DNA integrity was assessed by 1 % agarose gel electrophoresis. DNA quality and concentration of the extracts were determined by using the NanoDrop 2000c Spectrophotometer (Thermo Scientific).

Real time qPCR analysis of fecal predominant bacteria

Real time qPCR assay was performed with standard procedure as said above. Specific primer pairs used to detect Bifidobacterium, Lactobacillus, Escherichia coli, Enterococcus spp and all bacteria are based on 16S rRNA gene sequences according to previous studies [19, 20] and shown in ▶ **Table 1**. The reaction condition for PCR amplification was as follows: 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Each assay was performed in triplicate in the same run. The copy number of the target DNA was calculated by comparing with a standard curve made by a 10-log-fold diluting standard genomic DNA. Bacterial numbers were presented as log 10 bacteria per gram of feces (wet weight).

Statistical analysis

Data are presented as the mean ± standard deviation (SD) (normally distributed) or median (interquartile range [IQR]) (not normally distributed). The non-normally distributed variables were log transformed to achieve a normal distribution prior to analysis. Comparisons of quantitative variables between groups were performed by Student's t test. All statistical analyses were performed using SPSS 17.0 (SPSS Inc, Chicago, IL). A P value of < 0.05 was regarded as statistically significant.

Results

Body weight gain and fat mass development after HFD feeding and berberine administration

As shown in ▶ **Table 2**, compared with NCD-fed rats (NC group), obesity was induced in rats fed a HFD for 28 weeks (HF1 group) (BW: 503.2 ± 41.4 g vs. 609.0 ± 66.9 g, $P < 0.001$). Moreover, HFD increased the accumulation of VFM in rats compared with the NC group (47.9 ± 20.3 g vs. 13.3 ± 8.2 g, $P < 0.05$). Rats in the HF1 group also had a higher VF/BW ratio than rats in NC group (0.07 ± 0.02 vs. 0.03 ± 0.02, $P < 0.05$). But co-administration of berberine at 200 mg/kg for 8 weeks had no significant effects on BW, VFM or the VF/BW ratio in HFD-fed obese rats.

▶ **Table 2** Comparison of metabolic and biochemical parameters for rats fed different diets with or without berberine treatment.

Parameters	NC group Week 28 (n = 10)	HF1 group Week 28 (n = 10)	HF2 group Week 36 (n = 10)	HF + BBR group Week 36 (n = 10)
BW (g)	503.20 ± 41.40	609.00 ± 66.90 * *	612.20 ± 60.70	593.20 ± 44.90
VFM (g)	13.30 ± 8.20	47.90 ± 20.30 *	44.10 ± 19.10	43.50 ± 19.80
VF/BW	0.03 ± 0.02	0.07 ± 0.02 *	0.07 ± 0.02	0.07 ± 0.03
LW (g)	13.10 ± 4.40	20.30 ± 8.30 *	22.40 ± 4.00	16.60 ± 3.40#
FBG (mmol/L)	5.55 ± 0.80	7.50 ± 0.94 * *	8.20 ± 0.60	7.00 ± 0.60#
FINS (ng/ml)	1.94 (1.49-2.55)	2.57 (1.59-3.78) *	2.7 (2.1-5.4)	2.0 (0.9-2.9) #
HOMA1-IR	10.19 (7.40-14.50)	15.01 (10.62-28.31) *	20.3 (14.2-44.6)	12.3 (6.4-19.4) #
TC (mmol/L)	1.66 ± 0.34	1.88 ± 0.38	2.07 ± 0.31	1.62 ± 0.21
TG (mmol/L)	0.50 (0.40-0.89)	1.15 (0.70-1.70) *	1.39 (0.66-1.64)	0.66 (0.38-0.80) #
HDL-C (mmol/L)	0.63 ± 0.24	0.57 ± 0.32	0.40 ± 0.12	0.45 ± 0.19
LDL-C (mmol/L)	1.52 ± 0.13	1.63 ± 0.28	1.65 ± 0.25	1.30 ± 0.14#
Plasma endotoxin (EU/ml)	0.08 ± 0.04	0.21 ± 0.12 * *	0.32 ± 0.09	0.15 ± 0.07#
Lobular inflammation (0-3)	0.00 + 0.00	1.80 ± 0.50 * *	2.80 ± 0.42	0.50 ± 0.53##

BW, body weight; HF + BBR group, high-fat diet (36 weeks) + berberine (8 weeks); FBG, fasting blood glucose; FINS, fasting insulin; HDL-C, high-density lipoprotein cholesterol; HF1 group, high-fat diet (28 weeks); HF2 group, high-fat diet (36 weeks); HOMA1-IR, homeostasis model assessment 1 to estimate insulin resistance; LDL-C, low-density lipoprotein cholesterol; LW, liver weight; NC group, normal control diet (28 weeks); TC, total cholesterol; TG, triglyceride; VFM, visceral fat mass; VF/BW, the visceral fat to body weight ratio. * $P < 0.05$ vs. that of NC group; * * $P < 0.001$ vs. that of NC group; # $P < 0.05$ vs. that of HF2 group; ## $P < 0.001$ vs. that of HF2 group.

Berberine reduced FBG and improve insulin sensitivity in HFD-fed obese rats

After consuming a HFD for 28 weeks, rats in the HF1 group exhibited significantly elevated FBG, fasting insulin and HOMA1-IR values compared with rats in the NC group (▶ **Table 2**). Berberine treatment for 8 weeks significantly decreased FBG, fasting insulin and HOMA1-IR values compared with the HF2 group (▶ **Table 2**).

Berberine improved lipid profiles in HFD-fed obese rats

As shown in ▶ **Table 2**, rats in the HF1 group had higher TG levels than rats in the NC group, but there were no significant differences in TC, HDL-C and LDL-C levels between the two groups. Treatment with berberine for 8 weeks significantly lowered TG and LDL-C levels compared with the HF2 group, while berberine had no significant influence in TC or HDL-C levels.

Structural changes in the liver and intestine

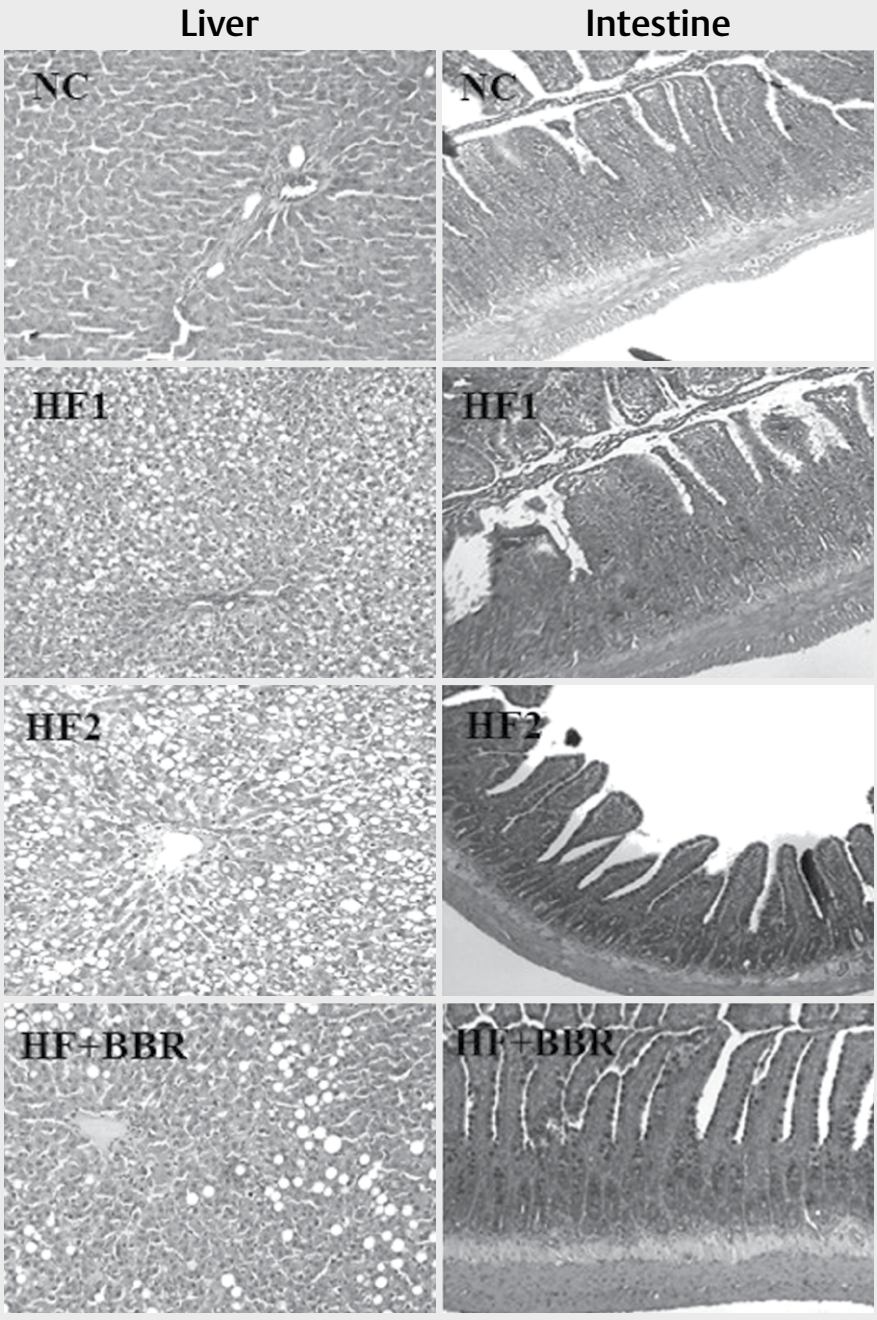
Rats in the HF1 group had significantly higher liver weights than rats in the NC group (▶ **Table 2**). Average liver weight decreased in the HF + BBR group compared with the HF2 group (▶ **Table 2**). As shown in ▶ **Fig. 1**, there were no abnormalities or obvious histopathological changes in the liver or intestinal samples of NC group rats based on H&E staining. Liver samples from the HF1 and HF2 groups showed moderate to severe steatosis, characterized by disordered hepatocyte cords, hepatocyte ballooning and lobular inflammation. And levels of lobular inflammation (0–3) in all groups were also investigated (▶ **Table 2**). The histological appearance of intestinal samples from the HF1 group demonstrated significant villous blunting and atrophy, discontinuity of the brush border and disarrayed epithelium, and these abnormalities were more obvious in the HF2 group. These changes in both the liver and intestine were significantly ameliorated in the HF + BBR group.

Quantification of the predominant gut microbiota by qPCR after HFD feeding and berberine administration

Relative population sizes of bacterial groups estimated by qPCR are presented in ▶ **Fig. 2**. Escherichia coli and Enterococcus spp. counts were both significantly higher in the HF1 group than in the NC group, while no significant differences in the counts of total bacteria, Bifidobacterium or Lactobacillus were observed between the two groups (▶ **Fig. 2a**). The abundance of Lactobacillus was significantly enriched by 8-week berberine treatment in the HF + BBR group (▶ **Fig. 2b**). A significant decrease of Bifidobacterium and increase of Escherichia coli counts were observed after prolonged HFD feeding for another 8 weeks in the HF2 group (▶ **Fig. 2b**). There was no significant difference in total bacterial count between the HF2 and HF + BBR groups (▶ **Fig. 2b**). Compared with the HF2 group, Bifidobacterium and Lactobacillus counts were significantly higher in the HF + BBR group, while Escherichia coli and Enterococcus spp. counts were significantly lower (▶ **Fig. 2b**).

Effect of berberine on LPS/TLR4/TNF-α signaling and IRc/IRS-1 expression in the liver

To determine if changes in the gut microbiota induced by berberine treatment contributed to reduced metabolic endotoxemia and associated inflammatory responses in the liver, we investigated plasma LPS concentrations and levels of hepatic TLR4/TNF-α expression. As shown in ▶ **Table 2**, rats in the HF1 group showed a significant increase in plasma LPS concentration compared with the NC group. Plasma LPS levels were significantly lower in the HF + BBR group than in the HF2 group. Consistent with changes in LPS concentration, rats in the HF1 group had elevated hepatic TLR4 and TNF-α mRNA (▶ **Fig. 3a**) and protein (▶ **Fig. 4**) expression compared with the NC group. Rats in the HF + BBR group showed sig-

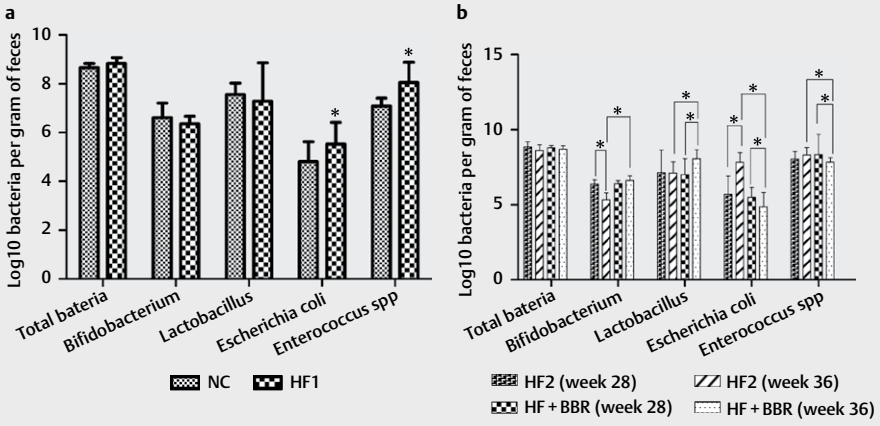


▶ **Fig. 1** Hematoxylin and eosin (H&E) staining of liver and intestine samples from rats fed different diets. **a** NC, normal control diet (28 weeks); **b** HF1, high-fat diet (28 weeks); **c** HF2, high-fat diet (36 weeks); **d** HF + BBR, high-fat diet (36 weeks) + berberine (8 weeks). Magnification: × 400.

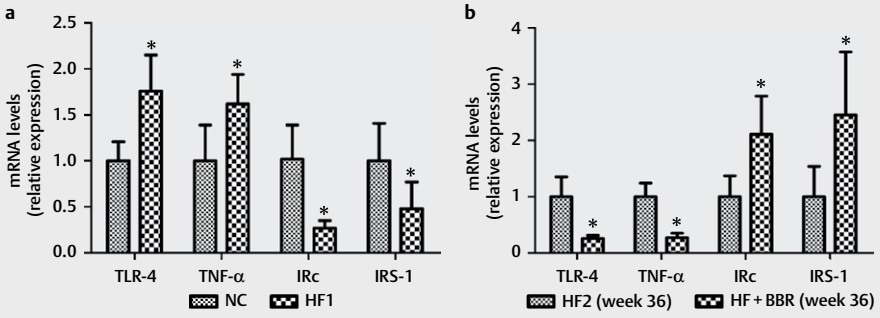
nificantly reduced hepatic TLR4 and TNF-α mRNA (▶ **Fig. 3b**) and protein (▶ **Fig. 4**) expression compared with the HF2 group. Previous studies have reported that TNF-α overexpression plays an important role in the development of obesity-related insulin resistance [8, 28]; therefore, we measured IRc and IRS-1 mRNA in the liver. Rats in the HF1 group exhibited obvious decreases in IRc and IRS-1 mRNA (0.27- and 0.48-fold, respectively) compared with the NC group (▶ **Fig. 3a**). Significant increases in IRc and IRS-1 mRNA (2.11- and 2.45- fold, respectively) were observed in the HF + BBR group compared with the HF2 group (▶ **Fig. 3b**).

Discussion

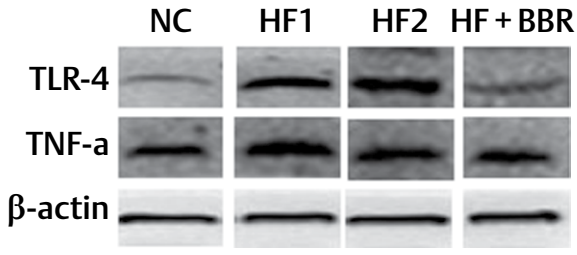
Compelling evidence has indicated that the gut microbiota is involved in HFD-induced inflammation and associated metabolic disorders such as insulin resistance and obesity [3, 21, 22]. Dysbiosis of the gut microbiota has been thought to initiate metabolic endotoxemia, which induces metabolic inflammation [3]. Modulating the gut microbiota through prebiotics and antimicrobial agents has been shown to alleviate insulin resistance and obesity in rodent models [23]. Thus, regulating the gut microbiota may represent a new therapeutic approach for combating insulin resistance and obesity.



▶ **Fig. 2** Structural changes of gut microbiota were estimated by real-time qPCR in rats. **a** NC group vs. HF1 group; **b** HF2 group (28 weeks) vs. HF2 group (36 weeks) vs. HF + BBR group (28 weeks) vs. HF + BBR group (36 weeks). NC, normal control diet (28 weeks); HF1, high-fat diet (28 weeks); HF2, high-fat diet (36 weeks); HF + BBR, high-fat diet (36 weeks) + berberine (8 weeks). * indicates statistical significance ($P < 0.05$).



▶ **Fig. 3** The determination of TLR4, TNF-a, IRc and IRS-1 mRNA levels in the liver of rats by real-time qPCR. **a** NC group vs. HF1 group; **b** HF2 group vs. HF + BBR group. NC, normal control diet (28 weeks); HF1, high-fat diet (28 weeks); HF2, high-fat diet (36 weeks); HF + BBR, high-fat diet (36 weeks) + berberine (8 weeks). * indicates statistical significance ($P < 0.05$).



▶ **Fig. 4** The determination of TLR4 and TNF-a protein levels in the liver of rats by Western blotting. NC, normal control diet (28 weeks); HF1, high-fat diet (28 weeks); HF2, high-fat diet (36 weeks); HF + BBR, high-fat diet (36 weeks) + berberine (8 weeks). * indicates statistical significance ($P < 0.05$).

Previous studies have shown that berberine has anti-hyperglycemic, hypolipidemic and anti-obesity effects in both humans and rodents [14, 24–26]. In this study, we reported that berberine treatment reduced triglyceride and LDL-C levels, alleviated insulin resistance, and protected against liver steatosis in HFD-induced obese rats. While these findings are consistent with previous studies [27, 28], we did not observe a significant BW reduction in the berberine-treated group as other studies have shown. Differences in dosages and treatment durations, and low rates of absorption after oral berberine administration might explain this discrepancy. Because berberine is poorly absorbed into the bloodstream and mainly acts topically in the gastrointestinal tract, it is speculated that berberine may exert anti-obesity and anti-insulin resistance effects by modulating the gut microbiota and intestinal inflammation [29].

Endogenous LPS, the toxic cell wall component of gram negative bacteria, is continuously produced upon death of these bacteria [30]. Gut microbiota dysbiosis, especially a reduction in the abundance of gut barrier-protecting bacteria such as Bifidobacterium and an enrichment of Gram-negative endotoxin producing bacteria such as Escherichia coli., may lead to impaired intestinal barrier integrity and increased plasma LPS [31]. Previous studies have linked the gut microbiota and endogenous LPS with low-grade systematic inflammation and insulin resistance [3, 31]. Selective enrichment of putative short-chain fatty acid-producing bacteria

by berberine treatment, which decreases LBP (a biomarker of circulating LPS), has been shown to prevent obesity and insulin resistance [15, 16]. Moreover, berberine has also been reported to attenuate insulin resistance and steatohepatitis by normalizing HFD-induced changes in the gut microbiota [27]. In our study, we found that berberine restored the levels of protective gut bacteria and lowered LPS levels. Therefore, our results indicated that berberine might prevent insulin resistance via modulating the gut microbiota and attenuating endotoxemia and inflammation.

Various biological pathways mediate the cross-talk between insulin resistance and the gut microbiota. Upon binding to its receptor, insulin activates receptor tyrosine kinases, which induce tyrosine phosphorylation of the insulin receptor substrates IRS-1, IRS-2 and IRS-3, activating downstream signaling [32]. Previous studies from our group have demonstrated that berberine prevents insulin resistance by modulating IR and IRS-1 expression in pancreatic β-cells and tyrosine phosphorylation of IR and IRS-1 in skeletal muscle in rats [33]. Consistent with the study by Kong et al. [34], our results suggested that berberine improved insulin sensitivity by increasing hepatic IRc and IRS-1 expression. Kong et al. showed that berberine up-regulated hepatic IRc expression through a protein kinase-C-dependent signal transduction pathway. However, our data indicated that berberine treatment reduced TNF-α expression by inhibiting LPS-induced TLR4 signaling. Furthermore, we showed that this inhibitory effect of berberine on the LPS/TLR4/TNF-α signaling pathway was due to berberine modulating the gut microbiota and attenuating endotoxemia. Considering the inhibitory effect of TNF-α on insulin signaling [6, 7], our results indicated that berberine reduces insulin resistance through a molecular mechanism that is at least partially attributable to its modulation of the gut microbiota and its anti-inflammatory effects combined with inhibiting LPS/TLR4/TNF-α signaling in the liver, which resulted in increased IRc and IRS-1 expression.

To our knowledge, this is the first study to demonstrate that berberine ameliorates insulin resistance by modulating the gut microbiota along with inhibiting hepatic LPS/TLR4/TNF-α signaling. Our findings are particularly noteworthy, considering that the liver is the first target of LPS-induced insulin resistance and plays a pivotal role in the development of T2DM and other metabolic diseases.

However, there are several limitations to this study that should be considered. First, as this was a preliminary study, we only investigated TLR4/TNF-α expression in the liver. TLR4/TNF-α expression in other tissues, e. g., adipose tissue, is also thought to play important roles in inflammation and insulin resistance, and thus, should be evaluated in future studies. Second, we did not assess the levels of phosphorylated IR or IRS-1. As the phosphorylated forms of these proteins are active, we cannot be sure whether altered expression of the non-phosphorylated forms of IR and IRS-1 detected in our study would affect insulin signaling. Third, we did not explore other critical molecules in the insulin signaling pathway such as Akt. A more comprehensive assessment of proteins involved in insulin signaling is warranted in future studies to more precisely demonstrate the effect of berberine on this pathway. Fourth, we did not investigate the effect of berberine on insulin sensitivity following TLR4 overexpression or TLR4 knockdown in liver cells or rats. Future studies designed to specifically test these models are needed to fully elucidate whether TLR4 is essential for berberine activity. Finally, we only quantified the relative abundance of target-specific bacteria by real-time qPCR, and it is difficult to detect new species by this method. To gain more insight into changes in the diversity of the gut microbiota after berberine treatment, a combination of real-time qPCR and other techniques like 16S rRNA pyrosequencing is needed in future studies.

Conclusions

In conclusion, we suggested that berberine treatment modulates the gut microbiota and alleviates HFD-induced insulin resistance in rats, at least partially along with attenuating plasma LPS levels and inhibiting LPS/TLR4/TNF-α-mediated inflammatory processes in the liver. Taken together, our results provide a new mechanical understanding of how berberine treatment regulates insulin sensitivity. Further exploratory study is required in determine the underlying signaling pathway.

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Conflict of Interest

No conflict of interest has been declared by the authors.

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