

Original Article

Berberine protected rats against adiposity induced by high-fat diets

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Abstract: Berberine (BBR) is the major active constituent of *Rhizoma coptidis*. The present study was carried out to investigate the effect of BBR on high fat rats and its possible mechanism. First, we used HE staining to detect the fat cells in epididymis tissues. To reveal the lipogenesis and lipolysis whether be affected by BBR, we used real time PCR to detect the mRNA expression level for lipogenesis genes FAS, ACC, SCD1 and SREBP1c, and lipolysis gene LPL, PPAR α , CPT1 and MCAD. Then we used the method ELISA to calculate the concentration of fat related proteins adiponectin and VLDL-C. We also used Western blot to detect the expression level of T-cadherin, adiponectin and adiponectin receptor 1 and 2. At last, western blot to detect the possible mechanism protein expression p-AMPK. Our data indicated that BBR could protect against adiposity by increasing the production of adiponectin and regulating the AMPK mechanism.

Keywords: Berberine, high fat, adiponectin, AMPK

Introduction

With the improvement of living standards, obesity has become an important disease to threaten human health. It is well known that increased body weight is a risk factor for the development of metabolic syndrome. Recently, obesity has also been shown to be associated with increased mortality for various cancers [1]. Natural products represent a rich reservoir of potential small chemical molecules exhibiting anticancer properties. Berberine (BBR), the major alkaloidal component of *Rhizoma coptidis* or cortex *phellodendri* and other medicinal materials [2]. Recent pharmacology research found that it had multiple pharmacological and biological activities including anti-arrhythmic, anti-inflammatory and anti-oxidative, extension of coronary vessels, reduction of hematic fat and glucose levels and anti-cancer [3, 4]. A number of animal experiments and clinical curative observation are confirmed that berberine can effectively reduce fat, glucose or weight. In high fat diet induced obesity mice model, BBR could significantly reduce the

mouse weight gain and serum glucose, triglyceride and total cholesterol levels, and had no adverse effects to normal diet mice [5]. In addition, in patients with type 2 diabetes mellitus (T2DM), BBR lowered fasting blood glucose (FBG), hemoglobin A1c, triglyceride and insulin levels [6]. We used randomized, double-blind, placebo-controlled and multiple-center trial in the treatment of type 2 diabetic patients with dyslipidemia, the results demonstrated that BBR displayed a potential anti-hyperglycemic and anti-dyslipidemia effect in these patients [7].

In this present study, our results indicate that berberine protect against adiposity in high fat diet mice accompanied with up-regulated p-AMPK expression without any obvious toxicity in HF and normal diet mice.

Materials and methods

Materials

Basal diet (AIN-93G diet) and the high-carbohydrate/high fat diet (66% basal diet; 15% lard;

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Table 1. Primers used in real-time PCR

Oligonucleotide	Sequences 5' to 3'
FAS-F	TGGTTCATTCCGTGACTG
FAS-R	TGTCCTTCGGTCTTTCC
ACC-F	TCCCAGTAAAGTTCAACAG
ACC-R	AAGGCAGTATCCATTCATC
SCD1-F	CAAAGAGAAGGGCGGAAAG
SCD1-R	CAAGAAGGTGCTGACGAAC
SREBP1c-F	CGCTACCGTTCCTCTATCAATG
SREBP1c-R	TCTGGTTGCTGTGCTGTAAG
LPL-F	GAAAGCCGAAGGCATGAG
LPL-R	CCCAAGTATCCCGAGAGTAG
PPAR α -F	ACCCACAGAGAACAGAAACAG
PPAR α -R	TTGGCTTTATGAGGAGGAGTC
CPT1-F	TTGGCAAGAGGAGTGAGAC
CPT1-R	CTGACTGGGTGGGATTAGAAG
MCAD-F	CCTGTGATTATTGCTGGAATG
MCAD-R	CTTCTCTGCTTTGGTCTTAATG

10% plantation white sugar; 6% casein and 3% yolk powder) were produced by Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai; China). SYBR Green PCR kit and first strand cDNA Synthesis kit were bought from Thermo. Primers for RT-PCR were synthesized by Shanghai Jierui Biological Engineering Co. Ltd. (Shanghai, China). Adiponectin and VLDL-C ELISA kit were bought from Wuhan Gene Biotech Co. Ltd (Wuhan, China). The antibodies for AMPK and p-AMPK were obtained from Santa Cruz Biotechnology (California, USA). The antibodies for adipoR1 (ab126611), adipoR2 (ab77612) and T-cadherin (ab167407) were obtained from Abcam (Cambridge, MA).

Rats model

40 male Wistar rats were obtained from Shanghai SLAC Laboratory Animal Co. Ltd. All rats used in the experiments were 8 wk old and weight matched, and housed 5 per cage with 12 h light-12 h dark cycles under controlled humidity (60 \pm 5%) and temperature (25 \pm 1°C). All procedures with rats were performed in accordance with the local (Shanghai) institutional guidelines and approval of the Animal Care Committees.

Rats were acclimatized to new environment for 1 week, and were then randomly divided into 4 groups. Group 1 (n = 10, named Normal Group) and group 2 (n = 10, named Normal + BBR

Group) were fed with basal diet while Group 3 (n = 10, named HF Group) and Group 4 (n = 10, named HF + BBR Group) were fed with the high-fat diet for 12 weeks. Every day of the last 8 weeks, rats of group 2 and group 4 were given a gavage of 380 mg BBR/kg body weight while rats of group 1 and group 3 were given placebo. Rats were anesthetised, blood and epididymal fat pads were collected for various experiments after drug intervention was finished.

Hematoxylin and eosin (H&E) staining

Epididymal adipose tissues were fixed in 10% formalin for 48 h at room temperature. Fixed tissues were embedded in paraffin and cut at 5 μ m for H&E staining. For H&E staining; the sections were deparaffinized; rehydrated; and stained with hematoxylin for 15 min. Sections were then rinsed in running tap water and stained with eosin for 1 min. HE stained sections were dehydrated and mounted.

Real-time PCR

Total RNA extraction; cDNA synthesis; and real-time PCR were performed as described previously [8, 9]. Briefly, total RNA was extracted from rats' epididymal adipose tissues using TRIzol reagent (Invitrogen). The purity and concentration of total RNA were measured by a spectrophotometer at 260 and 280 nm. Complementary DNA was synthesized from 1 μ g of total RNA using SuperScript II reverse transcriptase (Invitrogen). Real-time PCR was carried out with a Roche LightCycler 480 PCR system with SYBR Green Master Mix and gene specific primers (Roche Diagnostics; Indianapolis; USA). PCR primers for FAS; ACC; SCD1; SREBP1c; LPL; PPAR α ; CPT1 and MCAD sequences are listed in **Table 1**. Target gene expression was normalized to GAPDH gene expression.

ELISA

The concentrations of adiponectin and VLDL-C in rats' serum were determined by ELISA according to the manufacturer's protocol. Briefly, 50 μ l of each sample was added to co-star EIA/RIA plate and washed. After blocking with 2% bovine serum albumin, the plate was incubated with a mouse monoclonal antibody. The absorbance was measured at 450 nm by using a spectrophotometer.

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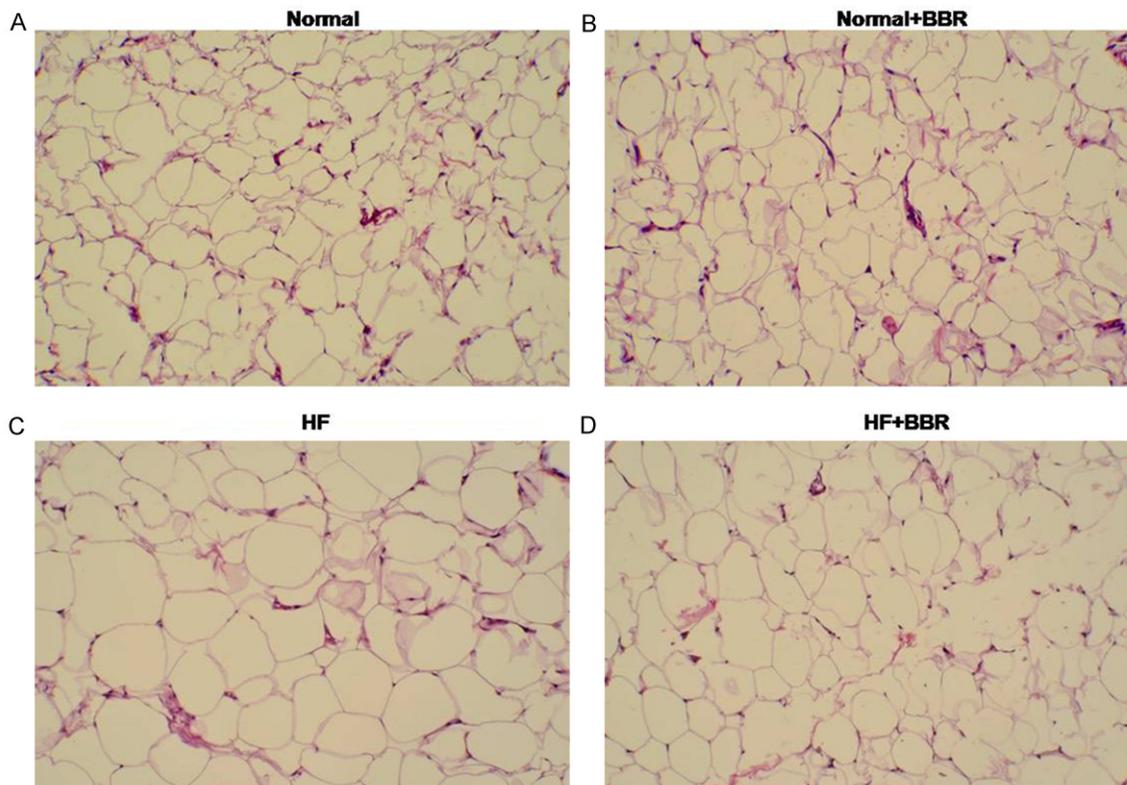


Figure 1. HE staining of epididymis fat tissue. A: The normal group; B: Normal + BBR group; C: HF group; D: HF + BBR group.

Protein extraction and western blot analysis

The protein extraction and western blot procedures were conducted. Briefly, total protein was isolated from rats' epididymal adipose tissues using RIPA lysis buffer. Protein concentrations were determined using Pierce BCA protein assay reagent (Pierce Biotechnology). Proteins were separated with 10% SDS-PAGE; and transferred onto PVDF membranes (Millipore Corp.; Billerica; MA; USA); blocked in 5% fat-free milk for 1 h; then incubated with primary antibodies (anti p-AMPK; AMPK; T-cadherin; adipoR1 and adipoR2) in 5% milk overnight at 4°C, which followed by incubation with secondary antibodies. Specific bands were visualized with enhanced chemiluminescence reagent and exposed to X-ray film. GADPH was used as an internal control.

Statistical analysis

All experimental data are presented as means \pm SD. Statistical significance was determined using the GraphPad Prism 5.0 software

(GraphPad, La Jolla, CA). Comparisons were made by 2-tailed Student's t tests. Effects were considered significant if $P < 0.05$.

Results

BBR intervention reduce the adipogenesis of rats fed with high-fat diets

To determine the effect of BBR in adipogenesis of rats fed with high-fat diets, histological analysis of epididymal adipose tissue was performed. White adipocytes are the master cell of epididymal adipose tissue, which have a unique structure in which nearly the entire cell volume is occupied by one large lipid droplet. As shown in **Figure 1**, Hematoxylin and eosin (H&E) staining exhibited that compared to normal diets, high-fat diets fed rats' epididymal adipose tissue mostly consists of large-sized white adipocytes in which almost the whole cell was occupied by one large lipid droplet, while cytoplasm was essentially undetectable. (**Figure 1A, 1C**), which suggesting the high-fat-diet model was established successfully. In contrast, the adi-

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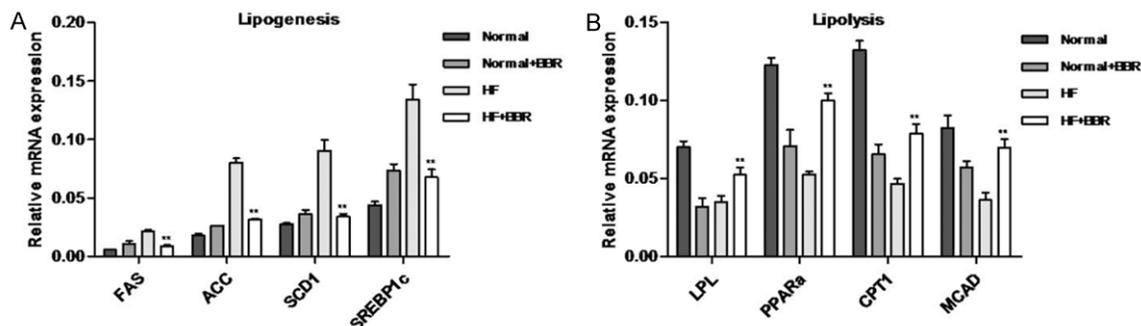


Figure 2. Real-time PCR to detect the lipogenesis and lipolysis related gene mRNA expression after treated with BBR. A: The lipogenesis gene FAS, ACC, SCD1 and SREBP1c mRNA expression were significantly decreased when compared to the HF group. B: The lipolysis gene LPL, PPARα, CPT1 and MCAD mRNA expression were increased after treated with BBR in HF rats.

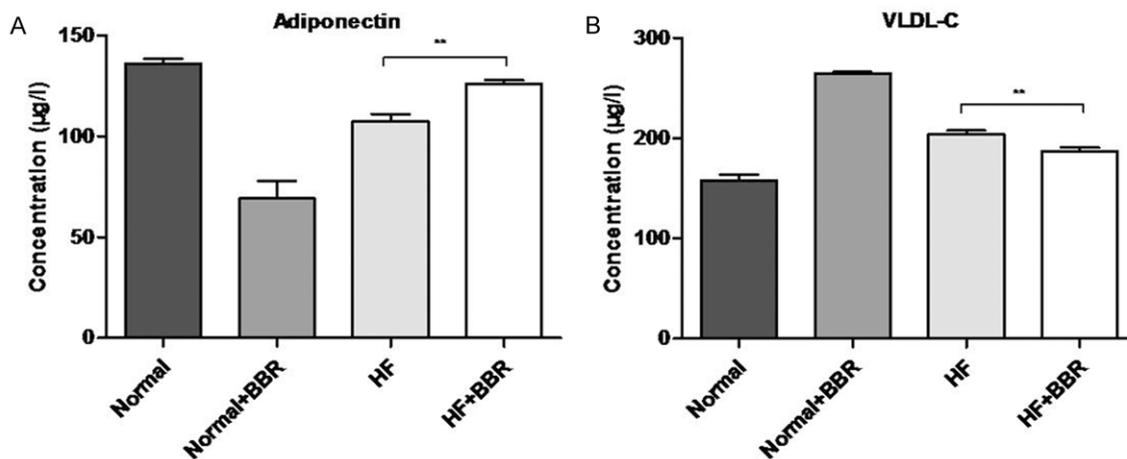


Figure 3. The effects of berberine on adiponectin and VLDL-C Elisa Result. A: The concentration of adiponectin was detected by Elisa; B: The concentration of VLDL-C was detected after the BBR treatment. Data are means \pm SD, n = 10. **Denotes significant difference compared with HF group ($P < 0.01$).

pose tissue of high-fat-diet rats intervened with BBR contained numerous small-sized adipocytes, which is among the normal and high-fat diets group (Figure 1A-D), indicating that BBR have an efficiency of reducing adipogenesis.

BBR up-regulated the expression of adipogenesis related genes and down-regulated the expression of lipolysis related genes

The expression level of adipogenesis and lipolysis related genes is indicators for adipogenesis. To determine the mechanism of BBR in reducing adipogenesis, quantitative real-time PCR was used to compare the mRNA expression of adipogenesis and lipolysis related genes in epididymal adipose tissues of every group rats. As shown in Figure 2, compared to HF group, BBR

intervention of high-fat-diet rats resulted in down-regulation of adipogenesis-related genes such as FAS; ACC; SCD1 and SREBP1c, while up-regulation of lipolysis-related genes such as LPL; PPAR α ; CPT1 and MCAD.

BBR up-regulated the concentration of adiponectin and VLDL-C in serum of high-fat-diet rats

Adiponectin, a 244-amino-acid-long polypeptide, which is involved in modulation of a number of metabolic processes including glucose-level regulation and fatty acid oxidation [10]. And its deficiency is associated with metabolic syndrome, obesity, type II diabetes and atherosclerosis. Level of adiponectin is inversely correlated with body fat percentage [11]. Very-low-

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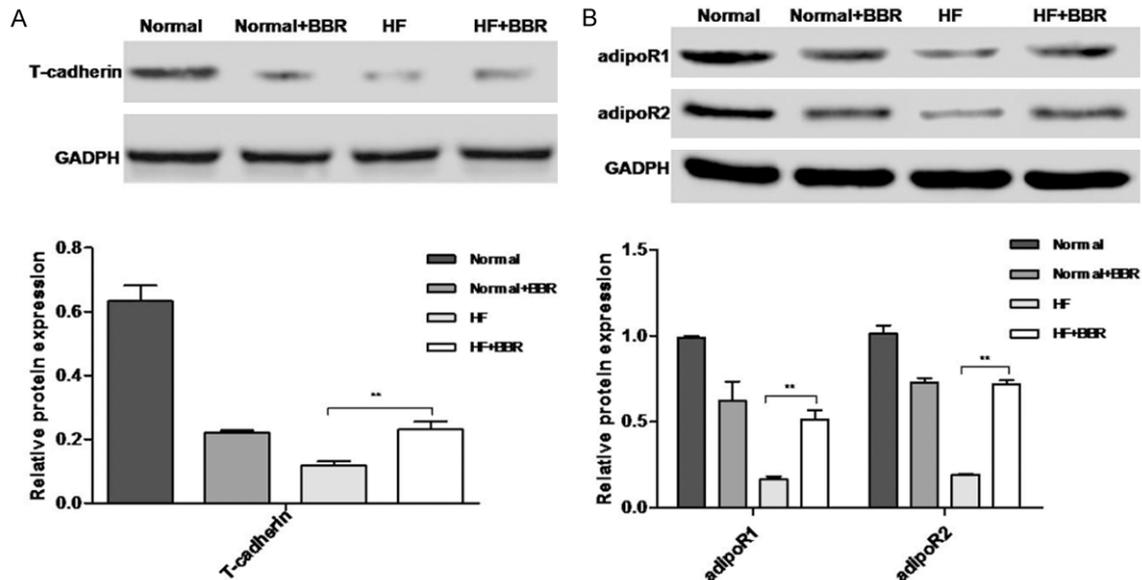


Figure 4. The protein expression of T-cadherin, adipoR1 and adipoR2 in Epididymis fat tissue. A: Western blot analysis of T-cadherin protein expression level. B: Western blot was carried out on the expression level of adipoR1 and adipoR2. Data are presented as means \pm SD, for each parameter, values bearing different superscript letters are different. ** $P < 0.01$, compared to the HF group.

density lipoprotein cholesterol (VLDL-C) is produced in the liver and released into the bloodstream to supply body tissues with a type of fat (triglycerides), is usually estimated as a percentage of your triglyceride value. After BBR intervention for 4 weeks, the concentrations of adiponectin and VLDL-C in the serum of every group rats were detected by ELISA. As shown in **Figure 3**, the adiponectin and VLDL-C concentrations in serum of BBR intervened HF group were significantly increased than that of HF group (** $P < 0.01$), indicating that BBR have an efficiency of promoting lipolysis.

BBR promoted T-cadherin, AdipoR1 and AdipoR2 expression in adipose tissue of high-fat-diet rats

Adiponectin plays a central role in antidiabetic and antiatherogenic adipokine, and adiponectin binds to three main receptors. Adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) are two receptors which have been identified with homology to G protein-coupled receptors. Obesity decreased expression levels of AdipoR1/R2, thereby reducing adiponectin sensitivity [12]. The other receptor, T-cadherin, is similar to the cadherin family. The receptors affect the downstream target AMP kinase, an important cellular metabolic rate control point.

The expressions of T-cadherin and AdipoR1/R2 in epididymal adipose tissue of every group rats were detected by Western Blotting. As shown in **Figure 4**, both the expressions of T-cadherin and AdipoR1/R2 were up-regulated in BBR intervened HF group compared to HF group.

BBR enhanced the activation of AMPK

AMP-activated protein kinase (AMPK) plays a key role as a master regulator of cellular energy homeostasis. AMPK can be directly phosphorylated on Thr172 by CAMKK2 in response to changes in intracellular calcium as occurs following stimulation by adiponectin. The net effect of AMPK activation include stimulation of hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis, inhibition of adipocyte lipolysis and lipogenesis. The phosphorylated AMPK (p-AMPK) is an indicator of the activation of AMPK pathway. To determine whether by activating AMPK signal pathway BBR enhanced lipolysis and reduced adipogenesis, the expressions of p-AMPK and AMPK in epididymal adipose tissue of every group rats were detected by Western Blotting. As shown in **Figure 5**, compared to HF group, the expression of p-AMPK was up-regulated in BBR intervened

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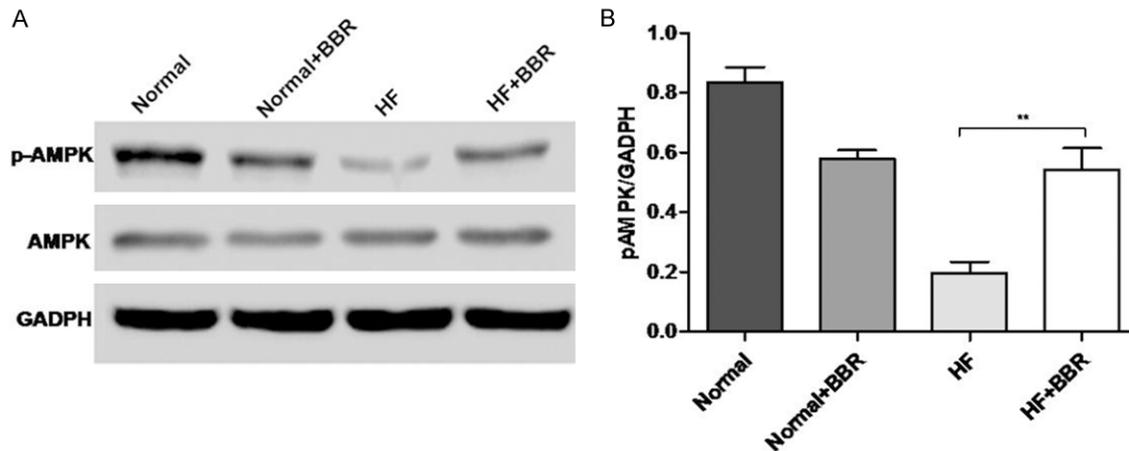


Figure 5. Western blot analysis of p-AMPK expression. A: Western blot showing the effect of berberine on p-AMPK levels in high fat; B: The relative expression of p-AMPK in relation to GADPH.

HF group, while the AMPK and GADPH were used as a loading control.

Discussion

Obesity is epidemic in much of world, affecting virtually of all socio-economic groups, irrespective of age, sex or ethnicity [13]. There is overwhelming evidence that obese individuals have a substantially higher risk of developing many diseases such as type 2 diabetes, hyperlipidemia and hypertension [14]. Thus the quest for possible compounds to aid in the treatment of obesity has intensified.

Berberine is usually used as an antibiotic drug for diarrhea, and for diabetic patients. BBR has also been used for hyperglycemia and dyslipidemia. In this study we used a high-fat diet mice model to verify the effects of BBR on important factors known to be involved in the development of obesity. Following BBR treatment in the mouse model, we observed the lipolysis genes mRNA levels were increased and lipogenesis genes levels were reduced in high fat diet group mice, showing berberine to be a potential natural compound for the treatment of adiposity.

Adiponectin, a 244 amino acid protein, is specifically and highly expressed in human adipose cells [15]. The physiological role for adiponectin may have anti-atherogenic, insulin resistance and anti-inflammatory properties [16, 17]. Furthermore, the adiponectin expression levels have been implicated to associate with the pathogenesis of obesity and type 2 diabetes

mellitus [18]. Very low density lipoprotein-cholesterol (VLDL-C) is produced in the liver and released into the bloodstream to supply tissues with a type of fat. High levels of VLDL-C have been associated with the development of plaque deposits on artery walls, which narrow the passage and restrict blood flow. ELISA result showed that BBR was significantly increased the concentration of Adiponectin and decreased VLDL-C concentration when compared to HF group. T-cadherin, adipoR1, adipoR2 are three receptors of adiponectin [19, 20]. The three proteins expressions were also increased. These results revealing that the effects of BBR protect against adiposity by regulating adiponectin.

However, as a therapeutic agent, the mechanisms of BBR in protecting against adiposity still need to be investigated. AMP-dependent protein kinase (AMPK), act as an intracellular energy sensor, regulates while body energy metabolism by activating glycolysis and fatty acid oxidation or inhibiting fatty acid and cholesterol synthesis, and AMPK might protect the body from metabolic diseases such as obesity and type 2 diabetes [21, 22]. Numerous reports have shown that BBR can bring about improvements in metabolic disorders by stimulating AMPK activity. For example, BBR reduces body weight and improves insulin sensitivity through AMPK activation in adipose tissue [23]. It has also been reported that BBR stimulates AMPK activity and fatty acid oxidation in hamsters fed a high-fat diet [24]. In this research, western blot result showed that the AMPK activation is

up-regulated after the BBR treated in HF group. These findings suggest that BBR displays beneficial effects in the treatment of obesity via stimulation of AMPK activity.

In conclusion, we have shown that BBR protect against adiposity through regulating adiponectin and VLDL-C concentration. The mechanism of action of BBR on obesity may be attributed to AMPK. From this study, we believe that berberine has excellent potential as an effective anti-obesity agent with no obvious toxicity.

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Disclosure of conflict of interest

None.

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