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Berberine Inhibits Gluconeogenesis in Skeletal Muscles and Adipose Tissues in Streptozotocin-induced Diabetic Rats via LKB1-AMPK-TORC2 Signaling Pathway*

Xiao-hu XU[1†], Qian HU[2†], Li-shan ZHOU[3], Li-jun XU[2], Xin ZOU[2], Fu-er LU[2], Ping YI[1#]
[1]*Department of Integrated Traditional Chinese and Western Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*
[2]*Institute of Integrated Traditional Chinese and Western Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*
[3]*Department of Integrated Traditional Chinese and Western Medicine, Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430016, China*

Summary: The effect and potential molecular mechanisms of berberine on gluconeogenesis in skeletal muscles and adipose tissues were investigated. After adaptive feeding for one week, 8 rats were randomly selected as the normal group and fed on a standard diet. The remaining 32 rats were fed on a high-fat diet and given an intravenous injection of streptozotocin (STZ) for 2 weeks to induce the diabetic models. The diabetic rat models were confirmed by oral glucose tolerance test (OGTT) and randomly divided into 4 groups (n=8 each), which were all fed on a high-fat diet. Berberine (3 g/kg per day) or metformin (183 mg/kg per day) was intragastrically administered to the diabetic rats for 12 weeks, serving as berberine group and metformin group respectively. 5-aminoimidazole-4-carboxamide1-β-D-ribofuranoside [AICAR, an agonist of AMP-activated protein kinase (AMPK), 0.5 mg/kg per day] was subcutaneously injected to the diabetic rats for 12 weeks, serving as AICAR group. The remaining 8 diabetic rats served as the model group, which was given a 0.5% carboxyl methylcellulose solution by oral gavage. Fasting serum insulin (FINS), OGTT as well as lipid parameters were tested by commercial kit. The protein levels of liver kinase B1 (LKB1), AMPK, phosphorylated AMP-activated protein kinase (p-AMPK), transducer of regulated CREB activity 2 (TORC2), phosphorylated transducer of regulated CREB activity 2 (p-TORC2), phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (G6Pase) in skeletal muscles and adipose tissues were examined by Western blotting. The results showed that berberine significantly decreased the body weight, plasma glucose, insulin levels, and homeostatic model assessment for insulin resistance (HOMA-IR) of diabetic rats compared with those in the model group. Meanwhile, the serum total triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) levels were markedly decreased and high-density lipoprotein cholesterol (HDL-C) level was significantly increased after the treatment with berberine. In addition, we found that berberine significantly increased the expression of p-AMPK and LKB1, while decreasing the p-TORC2 levels in skeletal muscles and adipose tissues. Moreover, the expression of PEPCK and G6Pase was significantly down-regulated after the treatment with berberine compared to the model group. It was suggested that the mechanism by which berberine inhibited peripheral tissue gluconeogenesis may be attributed to the activation of the LKB1-AMPK-TORC2 signaling pathway.
Key words: berberine; gluconeogenesis; skeletal muscle; adipose tissue; LKB1-AMPK-TORC2

Diabetes is a global public health crisis. It was reported that the prevalence of diabetes will increase by 54% and the annual deaths will climb by 38% in the United States between 2015 and 2030[1]. Similarly, it was estimated that more than 92 million Chinese adults had diabetes and another 148 million were prediabetes[2]. As a disorder of glucose metabolism, diabetes mellitus affects multiple organ systems and is associated with a variety of vascular and several nonvascular complications, such as diabetic eye

Xiao-hu XU, E-mail: hermanxu@163.com; Qian HU, E-mail: 2430249928@qq.com
†The authors contributed equally to this study.
#Corresponding author, E-mail: pyi219@163.com
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disease and diabetic nephropathy. Thus, diabetes causes a large financial burden on a family or society. Insufficient pancreas β cell function, ectopic fat accumulation, and insulin resistance are considered as the central point in the pathogenesis of diabetes[3]. In China, poor education, family history of diabetes, physical inactivity and overweight or obesity should account for this disease[4]. Metformin is the basic drug of antihyperglycemic therapy, which is generally the first-line drug used to treat type 2 diabetes mellitus (T2DM)[5]. However, the side effects of metformin are also obvious, such as gastrointestinal disorders (nausea, abdominal cramps, and diarrhea) and lactic acidosis. Recently, natural products and medicinal plants with antidiabetic potential have received more and more attention[6]. It has become a research hot-spot to seek reliable drugs to improve glucose and lipid metabolism and to prevent and control the occurrence and development of diabetes.

Berberine (molecular formula, $C_{20}H_{19}NO_5$; molecular weight, 353.36) is the main active component of an ancient Chinese herb *Coptis Chinensis*, which has been used to treat Xiao Ke (which means diabetes in Chinese) for thousands of years. Modern pharmacology research proves that berberine and metformin share many same characters in treating T2DM, obesity, cardiac diseases, tumor as well as inflammation[7, 8]. Metformin-mediated metabolism disturbance was proven to be the AMP-activated protein kinase (AMPK) activation[9]. Similarly, berberine was found to have antiobesity and antidiabetic effects through activation of AMPK and may exert a beneficial effect on pancreatic β-cell[10, 11]. What's more, our previous experiments proved that Huanglian jiedu decoction improved insulin resistance through elevating glucose transporter 4 (GLUT4) protein expression and translocation in adipose and skeletal muscle tissues, and berberine can inhibit gluconeogenesis via the liver kinase B1 (LKB1)-AMPK-transducer of regulated CREB activity 2 (TORC2) in liver tissues[12, 13].

Aberrant activation of hepatic gluconeogenesis and insulin resistance are the primary factors for increased hepatic glucose production and hyperglycemia in T2DM patients. Skeletal muscle is the dominating organ for glucose consumption. It plays an important role in regulating calorie balance which takes up approximately 75% of glucose consumption in the human body[14]. Meanwhile, skeletal muscle is associated with insulin resistance, which contributes to approximately 80% of insulin-stimulating glucose disposal[15, 16]. By strengthening skeletal muscle glucose uptake and inhibiting gluconeogenesis, the level of blood glucose will be reduced greatly. Obesity is characterized by the accumulation of adiposity. Statistics show that 60%–90% of all patients with T2DM are or have been obese[17]. Adipose tissue, one of the organs of glucose utilization, not only secretes adipokines regulating glucose and lipid metabolism as an endocrine organ, but also is a central component for whole-body energy homeostasis regulation[17, 18]. Adiponectin, secreted by adipose tissue, was reported to inhibit hepatic gluconeogenesis and stimulate the oxidation of fatty acids in skeletal muscles of sugarcane[18]. Increasing evidence has confirmed that the suppression of adipose lipolysis indirectly suppressed the hepatic gluconeogenesis and reduced blood sugar[19].

AMPK has been identified as a key regulator of whole-body energy balance, which also is a therapeutic target for the metabolic dysfunction in obesity and insulin resistance. When cellular energy levels are low, AMPK is activated to stimulate glucose uptake in skeletal muscles, fatty acid oxidation in adipose and other tissues, and reduce hepatic glucose production[20]. LKB1, as the upstream of AMPK, mediates phosphorylation of the AMPK α catalytic subunit at Thr172. Activated AMPK subsequently phosphorylates key proteins concerned with the regulation of hepatic gluconeogenesis and lipid metabolism, such as the decomposition of the CAMP-CREB-TORC2 transcriptional complex[21]. TORC2 is phosphorylated at Ser171, breaks away from the blinding of CREB and transports into the cytoplasm to inhibit gluconeogesis[22, 23]. So could berberine, the major component of huanglian jiedu decoction, inhibit gluconeogenesis and improve lipid metabolism in skeletal muscles and adipose tissues of the diabetic rat? And whether this effect is through targeting the LKB1-AMPK-TORC2 signaling pathway?

In this study, a high-fat diet and streptozotocin (STZ) tail vein injection were used to establish a diabetic rat model. Indicators of lipid and glucose metabolism were observed and LKB1-AMPK-TORC2 signals in skeletal muscles and adipose tissues were detected by Western blotting. We found that berberine inhibits gluconeogenesis in skeletal muscles and adipose tissues in STZ-induced diabetic rats via the LKB1-AMPK-TORC2 signaling pathway.

1 MATERIALS AND METHODS

1.1 Animal Care and Use Statement

Forty male Wistar rats (aging about 7–8 weeks, weighing about 200–230 g) were supplied by the Centers for Disease Control and Prevention (Wuhan, China) and housed in the animal experimental center of Tongji Medical College, Huazhong University of Science and Technology (China). The rats were maintained at ambient temperature (22°C±1°C) with 12-h light-dark cycles and free access to water and the standard rat diet. All experimental procedures were performed in accordance with the guiding principle for experimental animals (MSTPRC Directive of 1988, No. 88-2).

1.2 Chemicals and Experimental Drugs

STZ was produced by Sigma (USA). Berberine was provided by the National Institute for the Control of Pharmaceutical and Biological Products (China). Metformin was purchased from Shenzhen Vanda Pharmaceuticals (China), and 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR) was procured from the Beyotime Institute of Biotechnology (China).

1.3 Experimental Design

After 1 week of adaptation, 8 rats were randomly assigned to a normal control group and fed on the standard rat diet (normal). The remaining rats were fed on the high-fat diet (containing 67.5% standard laboratory rat chow, 15% lard, 15% sugar, 2% cholesterol, and 0.5% bile salts). After two weeks, all rats except the normal group fasted overweight. Next, these overweight rats were subjected to the tail vein injections with a dose of 30 mg/kg body weight of STZ (Sigma Chemical Co., USA) dissolved in 0.05 mol/L sodium citrate (pH 4.5) after 12-h fast for induction of the diabetic models. Two weeks after injection, impaired glucose tolerance (IGT) in rats was determined by oral glucose tolerance test (OGTT) (95% range of confidence was calculated according to the plasma glucose levels of normal rats). The rats with diabetes (i.e., rats with plasma glucose levels that were above the normal upper limit at two-time points or 20% greater than the normal upper limit at one-time point) were selected and a second injection was applied if rats were not diabetic. The diabetic rats were randomly divided into the following four groups (n=8 each). The diabetic model group (model) was given 0.5% carboxyl methylcellulose solution by oral gavage. Berberine-treated group (BBR or berberine) was given berberine solution (3 g/kg every day) by oral gavage. Metformin group (MET or metformin) was given metformin hydrochloride solution (183 mg/kg every day) by oral gavage. AICAR group (AICAR) was given the subcutaneous injection of AICAR solution (0.5 mg/kg, once a day). By the end of the 12 weeks, OGTT was carried out and the tail blood samples were tested by fasting insulin (FINS), and the experimental process is shown in fig. 1. Finally, after overnight-fasting, all rats were weighed and then anesthetized by 1% pentobarbital. Blood samples were obtained from the abdominal aorta and allowed to clot for 30 min at 4°C. After centrifuging at 3000 r/min for 15 min at 4°C, the serum was separated and stored at –80°C until examination. The skeletal muscle and adipose tissues were quickly excised and immediately frozen in liquid nitrogen and stored at –80°C until use. All procedures were approved by the Animal Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology.

1.4 OGTT

The rats were fasted overnight and given a single dose of 50% glucose solution. The glucose solution was administered by gavage at a dose of 2.2 g/kg body weight. The tail blood samples for glucose measurement were obtained from the tail vein at 0 h (before glucose loading), 1 h, and 2 h (after glucose loading) with the glucose oxidase method using a glucose monitor (LifeScan Milpitas, USA).

1.5 Measurements of Plasma Insulin and Lipids

FINS was measured by a radioimmunoassay kit (Northern Institute of Biotechnology, China). The homeostasis model assessment index (HOMA-IR) was calculated using the formula of fasting glucose (mmol/L) × fasting insulin (μIU/mL)/22.5. The serum total cholesterol (TC), total triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were determined by commercial reagents (Jiancheng Bioengineering Institute, China).

1.6 Western Blotting

The proteins of the skeletal muscles and adipose tissues were separately extracted, and the BCA method was used to measure the concentrations of proteins. The sample protein extractions (200 μg) were mixed with loading buffer, boiled for 5 min, and separated on 10% SDS-PAGE. The isolated proteins were finally added to the Marker protein at the end of the two terminals and electrophoretically transferred to nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk (5 mL) for 1 h at room temperature. Then the membranes were washed in TBST and incubated overnight with primary antibodies (LKB1, AMPK, p-AMPK, TORC2, p-TORC2, G6Pase, and

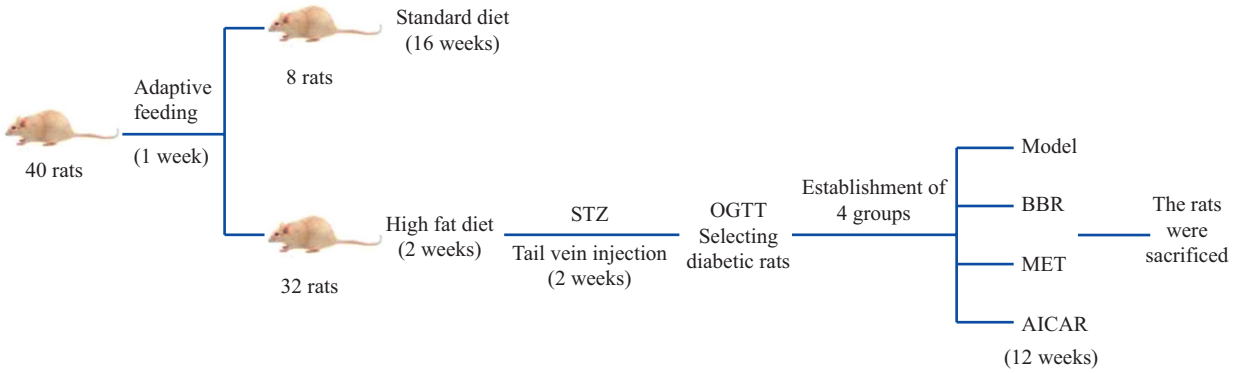

Fig. 1 The experimental process

PEPCK) at 4°C. After washing three times for 10 min each with TBST, secondary antibodies with infrared fluorescent labeling were incubated for 1.5 h at room temperature under the condition of avoiding light. After washing three times for 10 min each with TBST, the proteins were detected using the immunofluorescence luminous method by LI-COR Odyssey Infrared fluorescent scanner. Densitometry was used to quantify the bands obtained by Quantity One Software (Bio-Rad Company, USA). The antibodies to LKB1 (ab58786), AMPK (ab80039), p-AMPK (phosphor T172), PEPCK (ab70358), and G6Pase (ab83690) were purchased from the Abcam (UK), and TORC2 antibody p-TORC2 provided by ProteinTech Group, Inc. (USA). All the antibodies were purchased from Wuhan AntGene Biotechnology Co. Ltd. (China).

1.7 Statistical Analysis

The data are presented as mean ± standard deviations (SD). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett T3 test for data with equal variances not assumed. For data with equal variances assumed, ANOVA followed by LSD test was used. Correlation analyses were performed using the Pearson correlation coefficient. Calculations were performed using GraphPad Prism version 4.03 and SPSS 17.0 software. Statistical significance was defined as P below 0.05.

2 RESULTS

2.1 Effects of Berberine on Body Weight, OGTT, Insulin Levels, and HOMA-IR in Diabetic Rats

As shown in fig. 2, as compared with the normal group, the body weight, fasting and postprandial plasma glucose (FPG, 1hPG, 2hPG), FINS, and HOMA-IR were significantly increased in diabetic rats (P<0.05, P<0.01). After treatment with berberine, metformin, and AICAR in diabetic rats, the body weight, plasma glucose, insulin levels, and HOMA-IR were significantly decreased as compared with those in the model group (P<0.05, P<0.01).

2.2 Effects of Berberine on Plasma Lipid Profiles in Diabetic Rats

As shown in fig. 3, the diabetic rats exhibited severer dyslipidemia characterized in TG, TC, LDL-C and HDL-C levels than the normal control group (P<0.01). After treatment with berberine or metformin, the serum TG, TC, and LDL-C levels were markedly decreased and the HDL-C level was significantly increased compared to the model group. Similarly, treatment with AICAR also decreased the serum TG and LDL-C levels (P<0.05, P<0.01), and increased the HDL-C level (P<0.01), although there was no significant difference in TC level between the AICAR group and the model group (P>0.01). Therefore, berberine not only reduces blood glucose and improves insulin resistance, but also regulates lipid disorder.

2.3 Effects of Berberine on p-AMPK/AMPK Expression in Skeletal Muscles and Adipose Tissues in Diabetic Rats

To test whether berberine could improve glucose and lipid metabolism through AMPK-activated pathway, p-AMPK and total AMPK protein abundance was detected by Western blotting after treatment with berberine, metformin and AICAR (agonists of AMPK) in skeletal muscles and adipose tissues of diabetic rats.

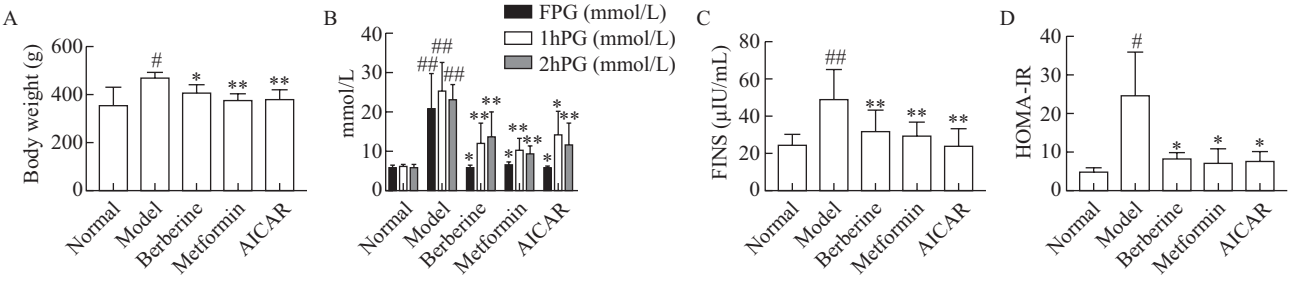

Fig. 2 Effects of berberine on body weight (A), OGTT (B), FINS (C) and HOMA-IR (D) in diabetic rats
$^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs.* control group; $^{*}P$<0.05, $^{**}P$<0.01 *vs.* model group

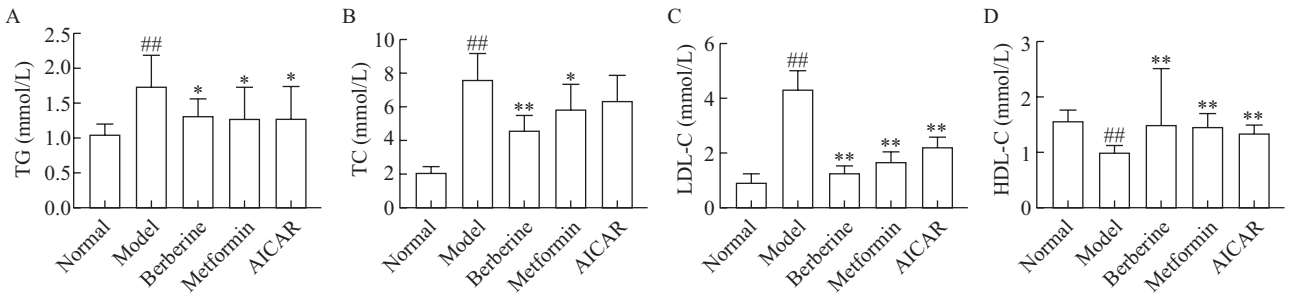

Fig. 3 Effects of berberine on plasma lipid profiles in diabetic rats
A: TG; B: TC; C: LDL-C; D: HDL-C. $^{\#\#}P$<0.01 *vs.* control group; $^{*}P$<0.05, $^{**}P$<0.01 *vs.* model group

As shown in fig. 4, in both skeletal muscles and adipose tissues, the expression of p-AMPK was down-regulated in diabetic rats as compared with that in the normal control rats (P<0.05). Berberine, metformin or AICAR treatment up-regulated the protein expression of p-AMPK compared to the diabetic model rats (P<0.05, P<0.01). The whole AMPK protein abundance was unchanged between these groups in skeletal muscles and adipose tissues.

2.4 Effect of Berberine on LKB1 Expression in Skeletal Muscles and Adipose Tissues in Diabetic Rats

To prove whether LKB1 regulates AMPK phosphorylation and participates in the energy metabolism in peripheral tissues, we examined the expression of LKB1 protein in skeletal muscles and adipose tissues in diabetic rats after treatment with berberine. The results showed that in both skeletal muscles and adipose tissues, the protein level of LKB1 in the model group was markedly lower than that in the normal control group (P<0.01). However, the expression of LKB1 in skeletal muscles and adipose tissues was significantly up-regulated after treatment with berberine, metformin and AICAR compared to diabetic model rats (P<0.01; fig. 5).

2.5 Effects of Berberine on p-TORC2/TORC2 Expression in Skeletal Muscles and Adipose Tissues in Diabetic Rats

The expression of p-TORC2 and total TOCR2 in skeletal muscles and adipose tissues in diabetic rats after treatment with berberine was detected. As shown in fig. 6, in both skeletal muscles and adipose tissues, the p-TORC2 level in the diabetic model rats was significantly higher than that in the normal control group (P<0.01). After treatment with berberine, metformin or AICAR, the p-TORC2 levels were significantly decreased compared to the model rats (P<0.05, P<0.01), but there was no significant difference in total

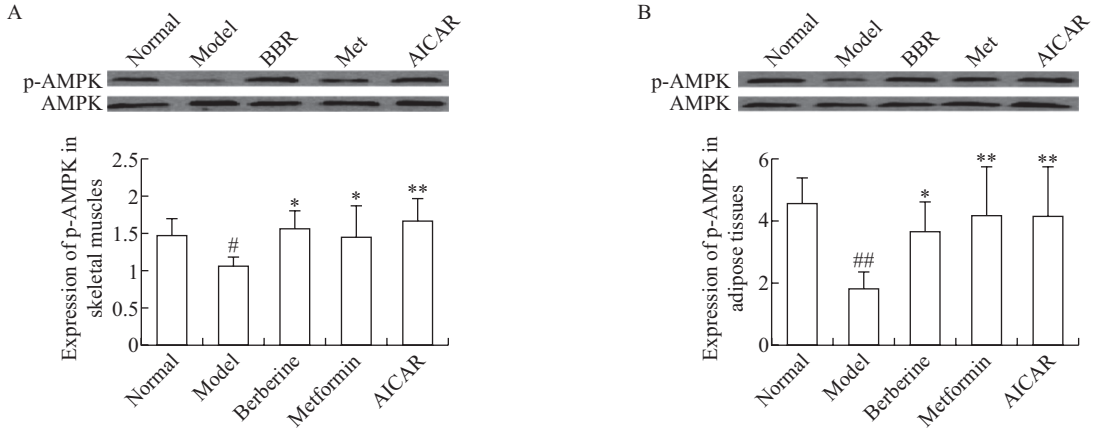

Fig. 4 Effect of berberine on p-AMPK/AMPK level

The p-AMPK/AMPK protein levels were tested by Western blotting in normal control group and diabetic model group, and diabetic rats treated with berberine (BBR), metformin (Met) and AICAR. A: skeletal muscles; B: adipose tissues. $^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs.* control group; $^{*}P$<0.05, $^{**}P$<0.01 *vs.* model group

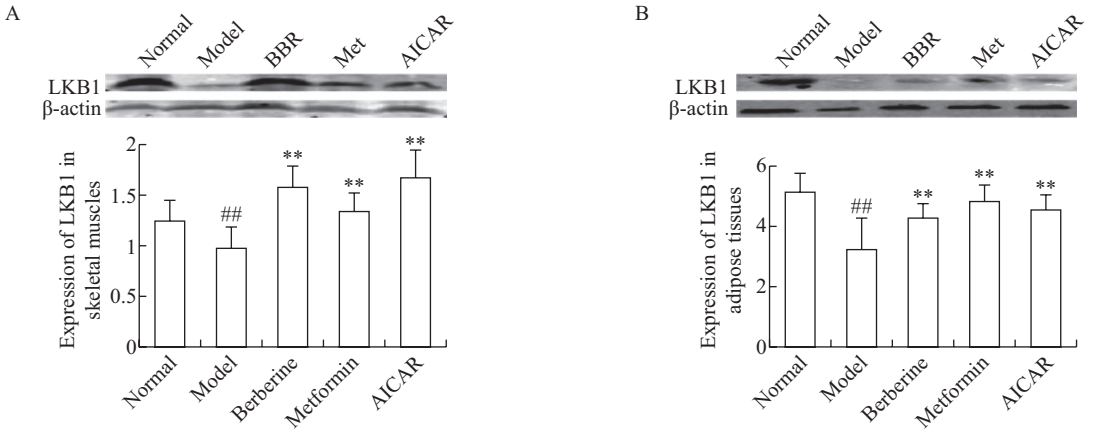

Fig. 5 Effect of berberine on LKB1 level

The LKB1 protein level was tested by Western blotting in normal control group, diabetic model group, and diabetic rats treated with berberine (BBR), metformin (Met) and AICAR. A: skeletal muscles; B: adipose tissues. $^{\#\#}P$<0.01 *vs.* control group; $^{**}P$<0.01 *vs.* model group

TORC2 protein level between different groups.

2.6 Effect of Berberine on PEPCK and G6Pase Expression in Skeletal Muscles and Adipose Tissues in Diabetic Rats

In this study, we explored the effect of berberine on PEPCK and G6Pase protein abundance in skeletal muscles and adipose in diabetic rats. As shown in fig. 7, in both skeletal muscles and adipose tissues, the expression of PEPCK was obviously increased in the diabetic model rats compared to the normal control group (P<0.01). After the treatment with berberine, the PEPCK protein abundance was significantly down-

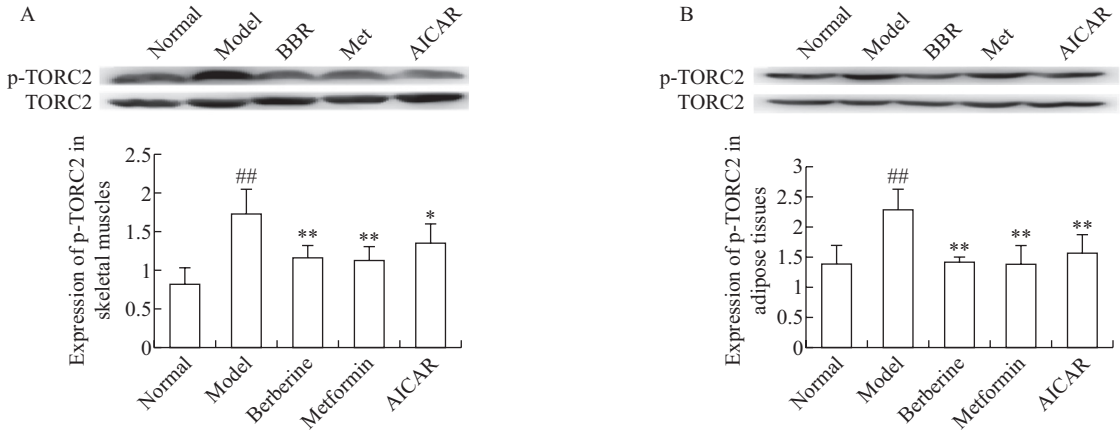

Fig. 6 Effect of berberine on p-TOCR2/TOCR2 level

The p-TOCR2/TOCR2 protein levels were tested by Western blotting in normal control group, diabetic model group, and diabetic rats treated with berberine (BBR), metformin (Met) and AICAR. A: skeletal muscles; B: adipose tissues. $^{\#\#}P$<0.01 *vs.* control group; $^{*}P$<0.05, $^{**}P$<0.01 *vs.* model group

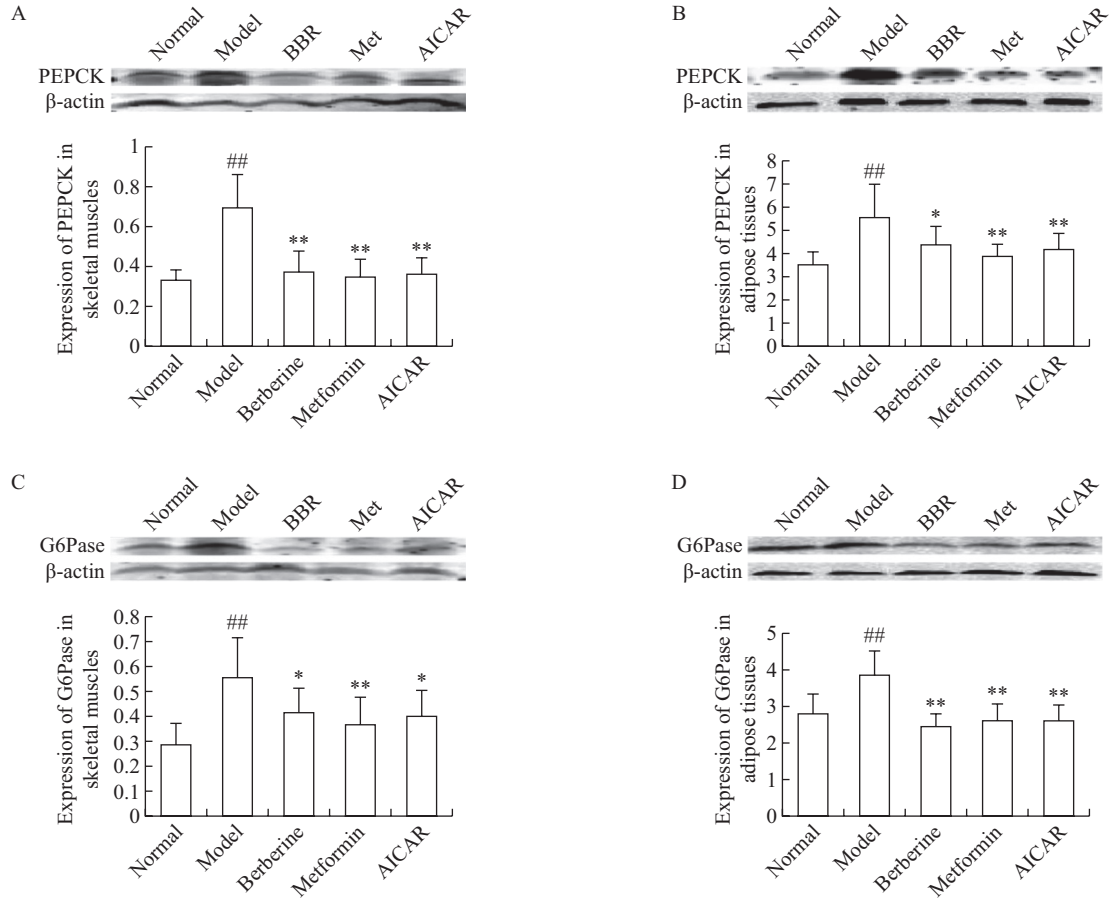

Fig. 7 Effect of berberine on PEPCK and G6Pase level

The PEPCK and G6Pase protein levels were tested by Western blotting in normal control group, diabetic model group, and diabetic rats treated with berberine (BBR), metformin (Met) and AICAR. A and B: PEPCK level; C and D: G6Pase level; A and C: skeletal muscles; B and D: adipose tissues. $^{\#\#}P$<0.01 *vs.* control group; $^{*}P$<0.05, $^{**}P$<0.01 *vs.* model group

regulated compared to the model group (P<0.01).

In skeletal muscles and adipose tissues, the expression level of G6Pase protein in the diabetic model group was markedly higher than that in the normal control group (P<0.01). After treatment with berberine or metformin or AICAR, the expression of G6Pase protein was down-regulated compared to the model group (P<0.05, P<0.01; fig. 7C).

3 DISCUSSION

Huang Lian and its major constituent berberine have a long history in treating "Xiao Ke" disease. Several studies have shown that berberine can improve glucose metabolism in diabetic rats by inhibiting hepatic gluconeogenesis[24, 25]. Based on the effects of berberine on glucokinase activity and hepatic gluconeogenesis, our previous study has also found that berberine inhibited hepatic gluconeogenesis in liver tissues of diabetic rats[12]. In this study, we further explored the effects of berberine on glucose metabolism and lipid metabolism in skeletal muscles and adipose tissues of diabetic rats. The results showed that the diabetic rats (model group) exhibited the characteristics of hyperglycemia, hyperinsulinemia, and hyperlipidemia. After treatment with berberine or metformin, the levels of FPG, 1hPG, 2hPG, FINS, HOMA-IR, TG, TC, and LDL-C were reduced to varying degrees, and HDL-C increased, although not returning to the normal levels. As the same with other researches, metformin, as the first-line drug against T2DM, has the best effect on weight loss, postprandial blood glucose reduction and insulin resistance[6, 26]. Berberine, an extract of traditional Chinese medicine *Coptis Chinensis*, reduced fasting blood glucose, the body weight, postprandial blood glucose, fasting insulin and insulin resistance of diabetic rats to varying degrees. More interesting, AICAR (AMPK agonist) group also showed the same trend with berberine and metformin in glucose metabolism and lipid metabolism, indicating that the AMPK seems to play an important role in the diabetes metabolism.

AMPK is a heterotrimeric protein composed of a catalytic α subunit and regulatory β and γ subunits. It is an important molecule that regulates liver functions, including gluconeogenesis and lipogenesis. It has been reported that some antidiabetic drugs, such as metformin and rosiglitazone, increase insulin sensitivity through the AMPK pathway[27, 28]. Berberine could also inhibit hepatic gluconeogenesis and adipogenesis in the liver via the AMPK pathway[11, 29]. To explore the relationship between berberine and AMPK in skeletal muscles and adipose tissues, Western blotting was used to detect the expression of AMPK and p-AMPK in these tissues. The results showed that the level of p-AMPK was significantly lower in diabetic rats than in the normal control group. Berberine, metformin, and AICAR can increase the expression of p-AMPK in diabetic rats. There was no significant difference in the total AMPK expression level between the groups. We can infer that p-AMPK is a protective regulator of diabetes, and berberine can regulate AMPK expression in skeletal muscle and adipose tissues to gain benefits.

LKB1 is a 50-kDa serine/threonine kinase that phosphorylates and activates the catalytic subunit of AMPK at its T-loop residue Thr^{172}. It has been reported that in adult mouse liver tissue, LKB1 regulates the phosphorylation of AMPK, and serves as a rate-limiting switch controlling gluconeogenesis[19]. To further verify whether berberine affects the gluconeogenesis of skeletal muscles and adipose tissues through the LKB1-AMPK pathway, we detected the LKB1 expression in these tissues and found that the LKB1 level was significantly lower in diabetic rats, and berberine, metformin, and AICAR can up-regulate the LKB1 expression. It demonstrated that LKB1 may be involved in the phosphorylation of AMPK by berberine.

TORC2 is thought to be one of the downstream molecules of AMPK, and it is also a key factor affecting the gluconeogenesis[30]. Interestingly, it has been reported that TORC2 had a moderate protective effect against fasting hyperglycemia[31]. Deficient hepatic TORC2 emerges at a low level of glucose output and gluconeogenic gene expression in mice[32]. When TORC2 is phosphorylated by activation of AMPK, it will stimulate gluconeogenesis through decreasing CREB binding to several gluconeogenic genes in the liver[33]. Insulin could stimulate phosphorylation of TORC2 but glucagon dephosphorylates it[34]. Under fasting conditions, circulating concentrations of pancreatic glucagon will stimulate gluconeogenesis through increasing the expression of several gluconeogenic genes induced by TORC2[22]. Our results showed that the protein expression level of p-TORC2 in the the model group was significantly up-regulated as compared with the normal control group. Berberine, metformin, and AICAR had negative effects on the expression of p-TORC2 in both skeletal muscles and adipose tissues. This result indicated that the LKB1-AMPK-TORC2 signaling pathway also existed in skeletal muscles and adipose tissues, and berberine may improve gluconeogenesis by regulating this signaling pathway.

In addition to the sugars from food sources, liver glycogen and the sugars produced by gluconeogenesis are the main sources of blood sugar. Excessive gluconeogenesis produces large amounts of glucose, resulting in hyperglycemia. Therefore, it is an effective measure to inhibit gluconeogenesis and reduce endogenous glucose generation. The gluconeogenesis rate-limiting enzymes, G6Pase and PEPCK, are two key hepatic gluconeogenic genes. It was well

known that TORC2 mediates CREB-dependent transcription of peroxlsome proliferator-activated receptor-γ coactlvator-1α (PGC1α) and its subsequent gluconeogenic targets PEPCK and G6Pase. It was reported that berberine inhibited gluconeogenesis in liver by down-regulating PEPCK and G6Pase[13]. To examine the effect of berberine on the two glycogenic key enzymes in skeletal muscles and adipose tissues, we investigated the protein expression of PEPCK and G6Pase after treatment with berberine. The results indicated that the expression levels of PEPCK and G6Pase in skeletal muscles and adipose tissues were significantly up-regulated in diabetic rats, and berberine, metformin, and AICAR could inhibit the expression of PEPCK and G6Pase.

To sum up, berberine could alleviate hyperglycemia and hyperlipidemia up-regulate the protein expression of LKB1 and p-AMPK, and down-regulate the protein levels of p-TORC2, PEPCK, and G6Pase in the skeletal muscles and adipose tissues. The mechanism by which berberine inhibited peripheral tissue gluconeogenesis may be attributed to the activation of the LKB1-AMPK-TORC2 signaling pathway.

Conflict of Interest Statement

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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