Berberine improves glucose metabolism through induction of glycosylation

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Berberine is a botanical alkaloid in the roots and bark of several plants, including Coptis chinensis French, an ancient Chinese herb that has been used to treat diabetes for thousands of years in China. Berberine is the main active compound of the herb. In addition to its metabolic activities, berberine has well-established antimicrobial activities in the control of infection by bacteria, viruses, fungi, protozoans, and helminthes (8, 14). It is an over-the-counter drug for the treatment of gastrointestinal infections in China. In 1988, the hypoglycemic effect of berberine was found when berberine was used to treat diarrhea in diabetic patients in China (13). Since then, berberine has been used as an antihyperglycemic agent by many physicians in China. There are many clinical reports about the hypoglycemic action of berberine in Chinese literature.

Regarding the mechanism of berberine action, we found that berberine increased glucose metabolism in cultured cells, and this activity was comparable to that observed for metformin in 2002 (20). This activity was confirmed later in other studies, and AMPK was proposed to mediate the metabolic activities of berberine (2, 3, 9, 22). In 2006, it was reported that berberine was able to activate AMPK for inhibition of lipid synthesis in human hepatocytes (2). Berberine was reported to reduce body weight and improve glucose metabolism in animal models of metabolic syndrome (9). Berberine was found to induce phosphorylation of AMPK in 3T3-L1 adipocytes and L6 myotubes, and AMPK was proposed to explain the insulin-sensitizing effect of berberine (9). Activation of the AMPK pathway by berberine was also observed by two other groups (3, 22). Although AMPK is activated by berberine, the metabolic conditions that lead to this effect require further investigation. Berberine was shown to inhibit the citric acid cycle in isolated mitochondria (1, 11, 12). However, it is not clear whether this activity can be observed in living cells for AMPK activation.

In this study, the metabolic activity of berberine was examined in diabetic rats, and the action mechanism was investigated in cellular models. The result suggests that berberine stimulates glucose metabolism through induction of glycosylation. Activation of AMPK by berberine is related to an increase in the AMP/ATP ratio. Inhibition of glucose oxidation in mitochondria may contribute to the AMP/ATP ratio increase and AMPK activation.

RESEARCH DESIGN AND METHODS

Reagents. DMEM, 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, insulin solution, 2-deoxy-D-glucose, 3-O-methyl-D-glucopyranose, and cytochalasin B were purchased from Sigma Chemicals (St. Louis, MO). Fetal bovine serum (FBS) was purchased from Atalanta Biologicals (Lawrenceville, GA). Glucose color reagent was purchased from Raichem (San Diego, CA). 2-Deoxy-D-[3H]glucose and 3-O-methyl-D-[14C]glucose were purchased from PerkinElmer (Boston, MA). Antibodies to phospho-Akt (Thr208) and phospho-p70 S6 kinase (Thr389) were purchased from Cell Signaling Technology (Danvers, MA). Anti-goat IgG [horseradish peroxidase (HRP) conjugated], antibodies to Akt1/2, GLUT4, and phospho-IRS-1 (Tyr632) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody to phospho-IRS-1 (Ser307) was purchased from Upstate (Charlottesville, VA). Antibodies to α-tubulin and GLUT1 were obtained from Abcam (Cambridge, MA). HRP-conjugated anti-rabbit IgG or anti-mouse IgG were purchased from GE Healthcare UK (Buckinghamshire, UK). PVDF membrane for immunoblot was purchased from Bio-Rad (Hercules, CA). Protein assay kit was purchased from Bio-Rad (Hercules, CA).
from Pierce (Rockford, IL). Berberine for animal experiments was from the Materia Medica Factory of Meitian County, Guizhou Province, China. Berberine for in vitro experiments was from Sigma Chemicals (St. Louis, MO).

Animal experiments. Male Wistar rats (Experimental Animals Center of Chinese Academic, Shanghai, China), 2–3 mo old and ~240 g body wt, were housed individually in wire cages in a temperature-controlled room (22 ± 1°C) on a 12:12-h light-dark cycle. The animals were fed a high-fat diet (HFD; lard, 59% of calories) with free access to food and water. The animal protocol was approved by the institutional review board. After 6 mo on HFD, the animals were divided into control (n = 6) and berberine-treated (n = 6) groups. Berberine was administrated at 125 mg/kg twice a day at 1000 and 2200 by gastric gavage for 5 wk. Control rats were gavaged with an equal volume of vehicle (distilled water). After 5-wk treatment, an intraperitoneal glucose tolerance test (IPGTT) was conducted between 0900 and 1100 on 10-h fasted animals. Fasting blood was first collected by lacerating the tail vein before glucose was given at 2 g/kg body wt (40% glucose solution). Then blood samples were collected at 2 h. Fasting insulin was detected using an enzyme immunoassay kit. Insulin was measured by radioimmunoassay method. The glucose of the wells with cells was subtracted from the glucose of the blank wells to obtain the amount of glucose consumption.

The cells were cultured in 96-well tissue culture plates. When experiments were conducted, the normal culture medium was replaced by DMEM supplemented with 0.25% BSA. Then ATP and AMP contents of the cells were measured using high-performance liquid chromatography (HPLC) as described by Wynants et al. (17). In brief, the cells were lysed in 0.6 N HClO4 and neutralized with 1 N KHCO3. HPLC analysis was developed on an HPLC system (waters Delta 600, Waters, Milford, MA) consisting of a solvent delivery pump unit (waters 600), an autosampler (waters 717 Plus), and a UV-Vis diode array detector (waters 2996 Photodiode Array Detector, 190–800 nm). The system was controlled and analyzed with the Empower software system (waters Delta 600). Separation was carried out using an Atlantis T3 column (5.0 μm, 4.6 × 150 mm ID; waters) with guard column (4.6 × 20 mm ID; waters). The mobile phase consisted of an aqueous buffer mixture of acetonitrile and methanol (50:50, vol/vol) where 0.15 M phosphoric acid (10 ml of 85% H3PO4/liter) was added to pH 6.00 with ammonium hydroxide (4 ml; A) and a mixture of acetonitrile and methanol (50:50, vol/vol; B). The optimal mobile phase was set as below: 0–5 min, 100% A; 5–25 min, gradient to A/B = 90:10; the flow rate was set as 0.7 ml/min, and the wavelength was set at 259 nm. ATP and AMP appeared at 9.6 and 18.1 min, respectively.

Lactate assay. The cells were cultured in a 24-well plate and treated with berberine and/or insulin in serum-free DMEM supplemented with 0.25% BSA. The lactate concentration in the medium was detected with a lactate assay kit from Biomedical Research Center, University of Buffalo (Buffalo, NY).

Statistical analysis. Data are presented as means ± SE. All experiments in cells were performed at least in triplicate. When a single comparison was performed, the significance of the differences between means was analyzed by Student’s t-test. When multiple comparisons were performed, the significance was analyzed by one-way ANOVA (SPSS 12.0). When a significant effect was found, differences between means were determined by Fisher’s least significant difference post hoc test. The P level was set at 0.05.
Berberine improved insulin sensitivity in dietary obese rats. In this study, dietary obese rats were used as an animal model of insulin resistance. At 6 mo on the HFD, blood glucose was significantly elevated in the Wistar rats in fasting and fed conditions. With 5-wk treatment by berberine, both fasting blood glucose (FBG) and postprandial blood glucose (PBG) were significantly reduced in the dietary obese rats (Fig. 1A). FBG was reduced from 5.74 to 5.39 mmol/l ($P = 0.035$). PBG was reduced from 10.4 to 7.6 mmol/l ($P = 0.039$), which is close to the normal glucose level. Fasting insulin level was decreased by 46% ($P = 0.045$; Fig. 1B). The insulin sensitivity was increased significantly by berberine, as indicated by a 48% reduction ($P = 0.036$; Fig. 1C) in HOMA-IR.

Berberine improved glucose metabolism in vitro. To investigate the mechanism of berberine action, glucose consumption was examined in several cell lines after berberine treatment. In 3T3-L1 adipocytes, berberine was found to increase glucose consumption in a dose-dependent manner (Fig. 2A). In the positive control, insulin (100 nmol/l) caused a twofold increase in glucose consumption in 24 h. A similar activity was observed for berberine in a dose-dependent manner. At concentrations between 5 and 20 μmol/l, berberine increased glucose consumption by 72.9 –113.7% in the absence of insulin ($P < 0.05–0.001$). In the presence of insulin, insulin-induced glucose consumption was further increased by berberine, which led to 41.2% more increase at 5 μmol/l ($P < 0.01$). This suggests that berberine and insulin had an additive interaction in the induction of glucose consumption in 3T3-L1 adipocytes. A similar effect of berberine was observed in C2C12 cells, in which glucose consumption was increased by 74.0% with berberine treatment at 20 μmol/l ($P < 0.01$; Fig. 2B). Interestingly, berberine exhibited a stronger effect over insulin in the muscle cells. Insulin by itself led to only a 47% increment of glucose consumption in the same condition. In L6 myotubes and H4IIE hepatocytes, berberine also increased glucose consumption in a dose-dependent manner (Fig. 2, C and D). At the concentration of 10 μmol/l, berberine led to 64.7 and 61.6% increments in glucose consumption in these two cell lines, respectively, which is comparable to the effects of insulin. However, berberine was unable to enhance insulin activity in L6 and H4IIE cells.

**RESULTS**

**Berberine improved insulin sensitivity in dietary obese rats.** In this study, dietary obese rats were used as an animal model of insulin resistance. At 6 mo on the HFD, blood glucose was significantly elevated in the Wistar rats in fasting and fed conditions. With 5-wk treatment by berberine, both fasting blood glucose (FBG) and postprandial blood glucose (PBG) were significantly reduced in the dietary obese rats (Fig. 1A). FBG was reduced from 5.74 to 5.39 mmol/l ($P = 0.035$). PBG was reduced from 10.4 to 7.6 mmol/l ($P = 0.039$), which is close to the normal glucose level. Fasting insulin level was decreased by 46% ($P = 0.045$; Fig. 1B). The insulin sensitivity was increased significantly by berberine, as indicated by a 48% reduction ($P = 0.036$; Fig. 1C) in HOMA-IR.

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**Berberine enhanced 2-deoxyglucose uptake in vitro.** Glucose utilization (consumption) is regulated at several steps, such as glucose uptake, glucose oxidation, glycogen synthesis,
and gluconeogenesis. Glucose uptake was investigated for berberine in 3T3-L1 adipocytes and L6 myotubes with 2-deoxy-D-[3H]glucose. In the absence of insulin, glucose uptake was elevated by 109.0–183.5% with berberine treatment in a dose-dependent manner in 3T3-L1 adipocytes (Fig. 3). In the presence of insulin, berberine (2 μmol/l) increased insulin activity by 38.9% (P < 0.01). In L6 cells, berberine-induced glucose uptake was much stronger than that of insulin. At 2 μmol/l berberine, glucose uptake was increased by 170% in the absence of insulin (P < 0.01; Fig. 3B). At 200 nmol/l insulin, glucose uptake was increased by only 80%. These results suggest that berberine is able to stimulate glucose uptake in both myotubes and adipocytes independently of insulin. In the presence of insulin, berberine enhanced insulin activity. Time course results showed that berberine was able to increase glucose uptake after 4-h treatment in 3T3-L1 adipocytes and L6 myotubes (P < 0.001; Fig. 3, C and D).

To study berberine’s effect on glucose transporter, 3-OMG uptake was conducted. The results suggested that berberine had less effect on 3-OMG uptake compared with 2-deoxyglucose uptake. Although 3-OMG uptake in 3T3-L1 adipocytes was enhanced by 52.8% with a high dose (10 μmol/l) of berberine in the absence of insulin (P < 0.01; Fig. 3E), little effect of berberine on 3-OMG uptake of L6 myotubes was observed (Fig. 3F).

Berberine-induced phosphorylation of AMPK. Effects of berberine on AMPK were detected in 3T3-L1 adipocytes and L6 myotubes (Fig. 4, A, B, and E). Phosphorylation of AMPK (Thr172) was induced by 2 μmol/l berberine in 3T3-L1 cells. In the presence of insulin, berberine enhanced insulin activity. Time course results showed that AMPK phosphorylation lasted for at least 16 h. These data suggest that berberine is able to stimulate phosphorylation of AMPK rapidly and maintain the elevated phosphorylation for a long time in cells.

The insulin-signaling pathway was examined in 3T3-L1 adipocytes and L6 myotubes in Western blotting (Fig. 4, A and B). In the positive control, insulin induced phosphorylation in IRS-1, Akt (Thr308), p70 S6 (Thr389), and ERK. IRS-1 phosphorylation was increased in both tyrosine (Tyr632) and serine (Ser636) residues by insulin treatment for 20 min. Berberine exhibited little effect on these signaling molecules in the presence or absence of insulin. No significant change was observed in GLUT1 and GLUT4 in berberine-treated cells. These data suggest that berberine does not directly influence the metabolic pathway of insulin and that its insulin-like activity may be independent of insulin pathway.

Berberine had no cytotoxicity. In this study, most of the cellular data were obtained with berberine treatment for 16 or 24 h. It is necessary to know whether berberine had toxicity in the cells. Thus, LDH cytotoxicity assay and protein assay were conducted to address this issue. In 3T3-L1 adipocytes, berber-
Berberine did not increase LDH release or reduce protein levels during the exposure time up to 24 h (Fig. 6A). In L6 myotubes, berberine treatment decreased LDH activity in a dose-dependent manner without altering the protein levels (Fig. 6B). These data suggest that berberine did not exhibit toxicity in our experimental system. It may protect plasma membrane of the cells.

**Berberine inhibited oxygen consumption in cells.** Activation of AMPK is often a consequence of an increase in the AMP/ATP ratio in the cells. Since ATP production is dependent on oxygen aerobic respiration in normal conditions, we examined oxygen consumption in 3T3-L1 adipocytes and L6 myotubes. Oxygen consumption was significantly reduced by berberine in a dose-dependent manner. In 3T3-L1 adipocytes, the oxygen consumption was decreased by insulin at 62.4% above the basal level. In the presence of berberine, the oxygen consumption was decreased by berberine even in the presence of insulin (Fig. 7A). The reduction was consistent in both types of cells. In 3T3-L1 adipocytes, the berberine activity was so strong that the basal oxygen consumption was reduced by 45.7% at 1 μmol/l berberine in the absence of insulin (P < 0.01). In the presence of insulin, the insulin-induced oxygen consumption was reduced by 63.5% (P < 0.001). In L6 myotubes, similar activities were observed for berberine at concentrations of 5–20 μmol/l (P < 0.05–P < 0.01; Fig. 7B). The time course study indicated that the cells grew well after being transferred to the BD Oxygen Biosensor Systems, as oxygen consumption of cells was increased in the control groups in a time-dependent manner, especially in L6 myotubes. On the other hand, oxygen consumption was not increased in the berberine groups (P < 0.01; Fig. 7, C and D), suggesting that oxygen consumption was inhibited by berberine. The difference between the control and berberine groups was observed as early as 2–3 h in the time course. The inhibition was enhanced with time of treatment in 3T3-L1 adipocytes. These data suggest that berberine may inhibit aerobic respiration in cells.

Effects of berberine on adenine nucleotide contents in vitro. The data above suggest that berberine may change adenine nucleotide contents in cells. To test this possibility, ATP and AMP contents were examined in 3T3-L1 adipocytes and L6 myotubes after berberine (5 μmol/l) treatment for 0.5 and 16 h. In 3T3-L1 adipocytes, ATP was decreased and AMP was increased by berberine. Thus, the ratio of AMP to ATP was elevated by 24.2% with short-term treatment and 33.3% with long-term treatment (P < 0.01; Fig. 8A). In L6 myotubes, AMP was increased significantly by berberine. Although ATP was also elevated, the AMP/ATP ratio was increased by 13.3% at 16 h with berberine treatment (Fig. 8B). These data indicate that berberine increases the ratio of AMP to ATP in adipocytes and myotubes, which may stimulate activation of AMPK.

**Berberine increased lactic acid release.** The data above indicate that aerobic respiration was reduced by berberine without loss of ATP content in cells. This result suggests that anaerobic respiration might be increased to compensate for the reduction in aerobic respiration. To test this hypothesis, release of lactic acid was measured in 3T3-L1 adipocytes and L6 myotubes. The lactic acid concentration was increased in the culture medium by berberine in a dose-dependent manner. In 3T3-L1 adipocytes, lactic acid release was increased with berberine by 47.4–163.8% in the absence of insulin and by 57.1–106.7% in the presence of insulin (P < 0.01–P < 0.0001; Fig. 9A). A similar change was observed in L6 myotubes. Lactic acid release was increased by 78.1% with 10 μmol/l berberine alone (P < 0.001; Fig. 9B). Time course results indicated that the berberine-induced lactic acid release was increased in a time-dependent manner from 4 to 24 h after berberine treatment (P < 0.01; Fig. 9, C and D). These data indicate that berberine is a potent stimulus for anaerobic respiration.

**DISCUSSION**

We observed that berberine was able to induce activation of AMPK. This is consistent with observations made by several other laboratories (2, 3, 9, 22). Berberine was reported to activate the AMPK pathway in HepG2 cells for inhibition of cholesterol and triglyceride synthesis (2). Activation of the AMPK-p38 pathway by berberine was proposed to be responsible for induction of glucose uptake in muscle cells (3, 9, 22). However, the role of the AMPK-p38 pathway is controversial.
for the metabolic activity of berberine. One study reported that berberine-induced glucose uptake was dependent on the AMPK-p38 pathway in L6 cells (3). In contrast, another report suggested that p38 was not involved in berberine activity in 3T3-L1 adipocytes (22). The significance of the AMPK pathway remains to be tested in the regulation of glucose metabolism by berberine.

Our study demonstrates for the first time that berberine inhibits oxygen-dependent glucose oxidation and enhances glycolysis in living cells. In cells, energy is transferred from

Fig. 5. Effects of berberine on phosphorylation of AMPK. A and B: 3T3-L1 adipocytes and L6 myotubes were treated with berberine for 16 h after serum starvation in cell culture medium with 0.25% BSA. Whole cell lysate protein was examined in a Western blot. C and D: cells were treated with berberine for a variety of times after serum starvation in 0.25% BSA-DMEM. Western blot was performed in cells to check phosphorylation and total protein levels of AMPK. Similar results were obtained from triplicate experiments, and a representative experiment is shown. Ratio of pAMPK to AMPK was quantified in 3 independent experiments per condition. Data are expressed as means ± SE; n = 3. Compared with control (0 μmol/l): *P < 0.05, **P < 0.01.

Fig. 6. Berberine had no cytotoxicity. Cells were starved in serum-free DMEM supplemented with 0.25% BSA and treated with berberine for 24 h. LDH concentrations in medium and protein level of cells were detected. A: berberine treatment had no effects on LDH release and protein level of 3T3-L1 adipocytes. B: berberine decreased LDH release of L6 myotubes in a dose-dependent manner. However, berberine had no effects on the protein level of L6 cells. Data are expressed as means ± SE; n = 3. Compared with control (0 μmol/l): *P < 0.05, **P < 0.01.
Glucose to ATP through aerobic respiration or anaerobic respiration (also called glycolysis). Aerobic respiration occurs in mitochondria and requires oxygen in the production of ATP from glucose. In contrast, glycolysis occurs outside mitochondria and produces ATP from glucose without consumption of oxygen. Glycolysis has a low efficiency in production of ATP and takes more glucose than aerobic respiration in production of the same amount of ATP. In this study, we observed that the oxygen consumption was reduced in living cells by berberine in a dose-dependent manner in cell culture. The reduction was observed in both adipocytes and myotubes. The reduction in oxygen consumption was associated with an increase in glycolysis, suggesting that berberine stimulates ATP production through glycolysis. Since more glucose is required to produce ATP in glycolysis, anaerobic respiration may be the primary mechanism by which berberine increases glucose utilization in cells.

The time course study suggests that glycolysis may lead to glucose uptake in cells treated with berberine. Although the increase in lactic acid and glucose uptake was observed at the same time (4 h after berberine exposure), the stimulation of glycolysis may occur first. Lactic acid was determined in the cell culture medium in this study. The increase in lactic acid should occur earlier inside the cells, since the secretion process delays the lactate increase in the culture medium. In this regard, the elevation in glucose uptake is likely a result of

![Image of Fig. 7: Berberine decreased oxygen consumption in cells.](http://ajpendo.physiology.org/)

![Image of Fig. 8: Adenine nucleotide contents in cells.](http://ajpendo.physiology.org/)
Increased demand for glucose in glycolysis. Glycolysis may be a result of mitochondrial inhibition. Inhibition of mitochondrial oxidation may contribute to AMPK phosphorylation induced by berberine. In the time course study, the AMP/ATP ratio was increased as early as 0.5 h by berberine treatment. AMPK phosphorylation was observed in both 3T3-L1 adipocytes and L6 muscle cells, suggesting the role of the AMP/ATP ratio in AMPK activation. In published studies, berberine activity was examined within 6 h of the AMPK activation. In the present study, the berberine effect was tested up to 16 h. Our data suggest that berberine-induced AMPK phosphorylation was detectable between 0.5 and 16 h. This activity of berberine is much stronger than that reported for adiponectin. Adiponectin-induced AMPK phosphorylation lasts only 15 min (19). Activation of AMPK normally leads to reversion of the AMP/ATP ratio by stimulation of ATP production in mitochondria; however, this did not happen in the berberine-treated cells. The AMP/ATP ratio remained at the high level for 16 h in the current study. The reason may be related to mitochondria inhibition by berberine, which is indicated by the persistent reduction in oxygen consumption in the cell culture. These data suggest that the long-lasting phosphorylation of AMPK in berberine-treated cells is a result of persistent mitochondria inhibition by berberine.

This study indicates that berberine has a weak effect on the glucose transporter as indicated by the 3-OMG data. Berberine is known to enhance glucose uptake (3, 7, 22). However, it is not clear whether the increase is due to glucose utilization or glucose transport across the cell membrane. It was reported that GLUT4 translocation was increased by berberine (7, 9). However, this activity is controversial, as it was not observed in another study (22). The difference might be related to the methods for the glucose uptake assays. In those published studies, the glucose uptake was measured with 2-deoxyglucose, which can be phosphorylated by hexokinase. The 2-deoxyglucose assay is not able to differentiate transportation from utilization when glucose uptake is increased. In the present study, 3-OMG, a nonphosphorylatable glucose analog (4), was used to test glucose transport in cells. The 3-OMG uptake was not altered in the L6 myotubes by berberine at any of the dosages tested. In the 3T3-L1 adipocytes, the 3-OMG uptake was increased by berberine only at a higher concentration (10 μmol/L). This fact suggests that berberine may have a weak effect on the glucose transporter at the high concentration (10 μmol/L). Data are expressed as means ± SE; n = 3. Compared with untreated cells (0 μmol/L): **p < 0.01, ***p < 0.001, ****p < 0.0001.

We did not observe a significant effect of berberine on the insulin-activated PI 3-kinase pathway. Our earlier study (20) indicated that berberine had an insulin-independent effect on glucose metabolism in vitro, but the mechanism was not clear. Two studies demonstrated that berberine had no effects on the insulin receptor-signaling pathway (3, 22). However, this conclusion was challenged by other reports, in which berberine was shown to activate the insulin-signaling pathway (7, 9). In the current study, we examined the protein level and phosphorylation status of major components (IRS-1, Akt, p70 S6 kinase, and ERK) in the insulin receptor-signaling pathway. Insulin-induced phosphorylation of the signaling molecules was not changed by berberine.

The cytotoxicity assay suggests that berberine has no toxicity in adipocytes and exhibits a protective effect in myotubes. One study suggested that berberine protected cardiac myocytes in the ischemia-reperfusion condition, since LDH and methylenedioxyamphetamine (MDA) leakage was reduced by berberine (21). Our data suggest that this effect of berberine is muscle specific, since it was not observed in adipocytes.

In summary, our data suggest that berberine improves glucose metabolism through induction of glycolysis. The increase in glycolysis is likely a consequence of inhibition of glucose oxidation in mitochondria. The elevated glycolysis may be a primary cause for upregulation of glucose uptake in muscle and adipocytes by berberine. Inhibition of mitochondrial oxidation by berberine may contribute to persistent elevation in the AMP/ATP ratio and long-lasting AMPK phosphorylation. Our data do not support the idea that berberine directly regulates signaling molecules in the insulin receptor-signaling pathway. Berberine represents a novel class of drug candidates for treatment of type 2 diabetes.
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