Original Article

Berberine, a Natural Plant Product, Activates AMP-Activated Protein Kinase With Beneficial Metabolic Effects in Diabetic and Insulin-Resistant States

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Berberine has been shown to have antidiabetic properties, although its mode of action is not known. Here, we have investigated the metabolic effects of berberine in two animal models of insulin resistance and in insulin-responsive cell lines. Berberine reduced body weight and caused a significant improvement in glucose tolerance without altering food intake in *db/db* mice. Similarly, berberine reduced body weight and plasma triglycerides and improved insulin action in high-fat–fed Wistar rats. Berberine downregulated the expression of genes involved in lipogenesis and upregulated those involved in energy expenditure in adipose tissue and muscle. Berberine treatment resulted in increased AMP-activated protein kinase (AMPK) activity in 3T3-L1 adipocytes and L6 myotubes, increased GLUT4 translocation in L6 cells in a phosphatidylinositol 3′ kinase–independent manner, and reduced lipid accumulation in 3T3-L1 adipocytes. These findings suggest that berberine displays beneficial effects in the treatment of diabetes and obesity at least in part via stimulation of AMPK activity. *Diabetes* 55:2256–2264, 2006

Obesity poses a serious health risk contributing to the increased prevalence of a host of other diseases including type 2 diabetes, hyperlipidemia, hypercholesterolemia, and hypertension (1,2). Peripheral insulin resistance, which is often associated with obesity, is one of the earliest detectable defects identified in individuals at risk of type 2 diabetes. For this reason, pharmacologic agents that overcome insulin resistance, so-called insulin-sensitizing agents, have received considerable attention. In recent years, several major insulin-sensitizing agents have been developed, including the thiazolidinediones (TZDs) (3) and metformin (4). Both of these agents are thought to have beneficial effects, at least in part, by activating the stress-activated kinase AMP-activated protein kinase (AMPK) (5,6). AMPK is activated under a variety of conditions that signify cellular stress, usually in response to a change in the intracellular ATP-to-AMP ratio. Active AMPK orchestrates a variety of metabolic processes, most of which lead to reduced energy storage and increased energy production. TZDs and metformin are thought to activate AMPK via discrete mechanisms; TZDs stimulate the proliferation of small adipocytes that secrete adipokines such as adiponectin, which have been shown to stimulate AMPK activity in muscle and liver cells (7). Conversely, it appears that metformin activates AMPK directly via an ill-defined mechanism (8). These studies emphasize the potential utility of targeting the AMPK pathway in the treatment of type 2 diabetes and obesity.

The use of natural products for the treatment of metabolic diseases has not been explored in depth despite the fact that a number of modern oral hypoglycemic agents such as metformin are derivatives of natural plant products (9,10). Although several traditional medicines have been reported to have antidiabetic effects (10), the molecular targets of such compounds have not been revealed, and a careful analysis of their mode of action in animal models has not been undertaken. In the present study, we have focused on berberine because this natural product has been reported in the Chinese literature and several recent studies (11–14) to have beneficial effects in human type 2 diabetes, although its mechanism of action is not known. Here, we show that in vivo administration of berberine has insulin sensitizing as well as weight- and lipid-lowering properties in both *db/db* mice and in high-fat–fed rats. Strikingly, berberine acutely stimulated AMPK activity in both myotubes and adipocytes in vitro, contributing to enhanced GLUT4 translocation in myotubes and reduced lipid mass in adipocytes. Based on these studies, we propose that berberine may have a major application as a new treatment for obesity and/or insulin resistance in humans.

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AICAR, 5-aminoimidazole-4-carboxamide riboside; AMPK, AMP-activated protein kinase; DMEM, Dulbecco's modified Eagle's medium; FAS, fatty acid synthase; MAPK, mitogen-activated protein kinase; PI, phosphatidylinositol; PPAR, peroxisome proliferator–activated receptor; TZD, thiazolidinedione; UCP, uncoupling protein; WAT, white adipose tissue.

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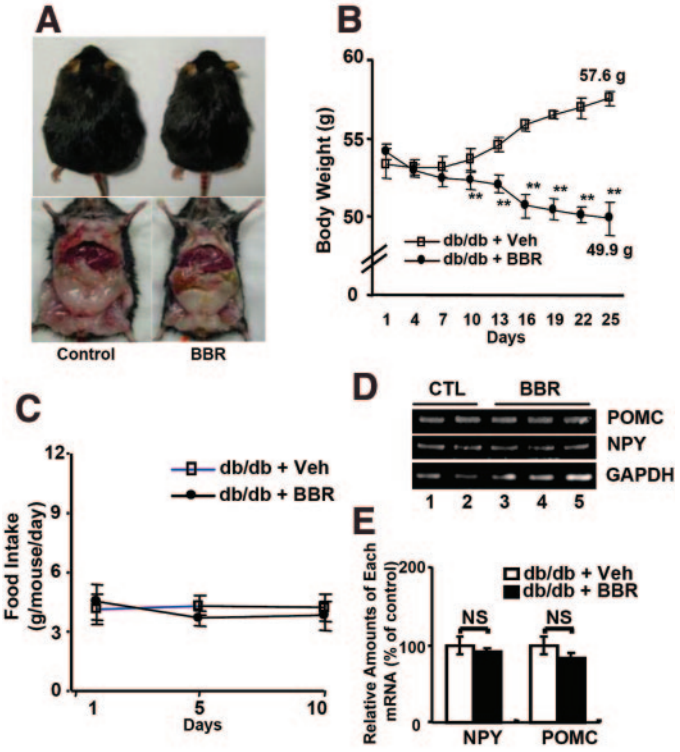


FIG. 1. Effects of berberine on body weight and food intake. *A*: Gross appearance of whole body and abdomen of vehicle (DMSO, Control, *left*)-treated or berberine (BBR, *right*; 5 mg · kg^{-1} · day^{-1})-treated *db/db* mice. *B*: Changes of body weight with or without berberine. *db/db* mice were treated with vehicle (□; $n = 16$) or berberine (●; $n = 17$) for 26 days. **$P < 0.001$. *C*: Effects of berberine on food intake. *db/db* mice were treated with vehicle (□) or berberine (●). *D*: Quantitative RT-PCR analyses of NPY and POMC genes in the presence or absence of berberine from whole brain. *E*: Relative amounts of each mRNA were normalized with amounts of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA and are indicated as fold changes. NS, not significant.

RESEARCH DESIGN AND METHODS

Mouse experiments. All experiments were approved by the Seoul National University Animal Experiment Ethics Committee. Obese and diabetic C57BLKS/J-*Lepr^{db}*/*Lepr^{db}* male mice were housed at 22 ± 2°C, 55 ± 5% relative humidity, with a light/dark cycle of 12 h. Food (Purina Mills) and water were given ad libitum. From 12 weeks of age, berberine (Wako, Osaka, Japan) was injected intraperitoneally (5 mg · kg body wt^{-1} · day^{-1}) into the mice for 26 days between 1400 and 1600. Thereafter, the brain, liver, right subcutaneous fat, epididymal fat tissues, interscapular brown adipose tissue, and skeletal muscle were dissected. After the dissection, the specimens were immediately frozen in liquid nitrogen and stored at −80°C.

Rat experiments. Wistar rats (250 g) supplied by the Animal Resources Center (Perth, Australia) were acclimatized in communal cages at 22°C, with a 12-h light 12-h dark cycle (lights on at 0600) for 1 week and had access to a standard chow diet (Gordon's Specialty Stock Feed, Sydney, Australia) and water ad libitum. Rats were then randomly assigned to receive either the standard chow diet as the control group (CH group) or a high-fat (60% calories as saturated fat) diet for 4 weeks (15). After 2 weeks of feeding, rats were randomly assigned to receive oral administration of either vehicle (0.5% methylcellulose) or berberine (380 mg · kg^{-1} · day^{-1}) by gavage for the last 2 weeks. Body weight and food intake were recorded daily. For euglycemic-hyperinsulinemic clamps (insulin infusion 0.25 units · kg^{-1} · h^{-1}), jugular and carotid cannulae were implanted 7 days previously, and animals were studied over 2 h in the conscious state after 5–7 h fasting, as previously described (16). All experimental procedures were approved by the Garvan Institute Animal Experimentation Ethics Committee and were in accordance with the National Health and Medical Research Council of Australia Guidelines on Animal Experimentation.

Cell culture and GLUT4 translocation assay. 3T3-L1 cells were cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine calf serum in an atmosphere of 10% CO_2. The differentiation of 3T3-L1 cells was induced as described previously (17). L6 myoblasts up to passage 15 were cultured in α-minimal essential medium supplemented with 10% heat-inactivated fetal calf serum (FCS) at 37°C in 10% CO_2. For differentiation into myotubes, cells were cultured in α-minimal essential medium supplemented with 2% heat-inactivated FCS at 37°C in 10% CO_2 and were maintained in this medium postdifferentiation. Myotubes were used for experiments 5–7 days after differentiation. To express HA-GLUT4 in L6 cells, myoblasts were infected with retrovirus as previously described (18) and differentiated as described above. The GLUT4 translocation assay was performed essentially as previously described (19).

RNA preparation and Northern blot analysis. Total RNA was prepared with TRIzol (Life Technologies-BRL) according to the manufacturer's instructions. Northern blot analyses were conducted in a previously described manner (17).

Measurement of triglycerides, free fatty acids, and cholesterol. Adipocytes were lysed, and the levels of intracellular triglycerides and free fatty acids were determined with commercial kits as described by the manufacturer (Sigma-Aldrich, St. Louis, MO). Blood samples were also collected from both

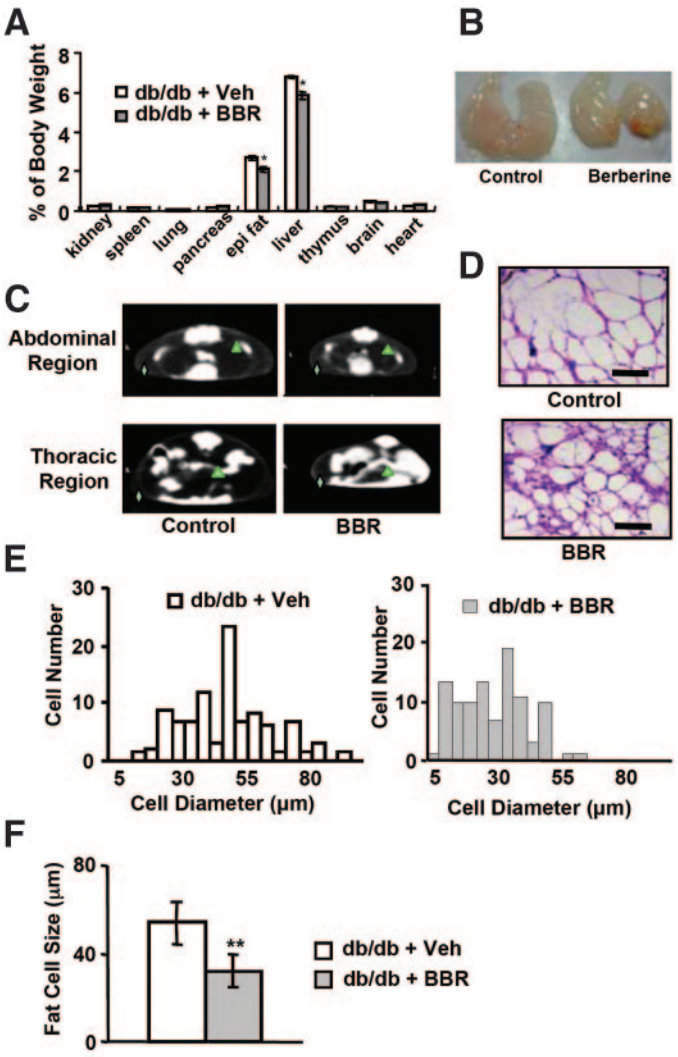


FIG. 2. Effect of berberine on white adipose tissue mass. *A*: The weights of all tissues from vehicle-treated mice (□; male, $n = 10$) and berberine-treated mice (▨; male, $n = 15$) normalized by total body weight. Data are mean ratios of the weight of each organ to total body weight (±SE). *$P < 0.002$. *B*: Appearance of epididymal WATs from vehicle- or berberine-treated mice. *C*: Computed tomography images of abdominal and thoracic regions of control (*left*) or berberine-treated (BBR) mice (*right*). Diamonds and triangles indicate subcutaneous fat and visceral fat, respectively. *D*: Histological analysis of subcutaneous WATs from control and berberine-treated mice. Samples were stained with hematoxylin and eosin and are photographed at ×100 magnification (bar = 50 μm). *E*: Distribution of fat cell sizes in WATs from control and berberine-treated mice. *F*: Average fat cell size. Values are means ± SE. **$P < 0.001$.

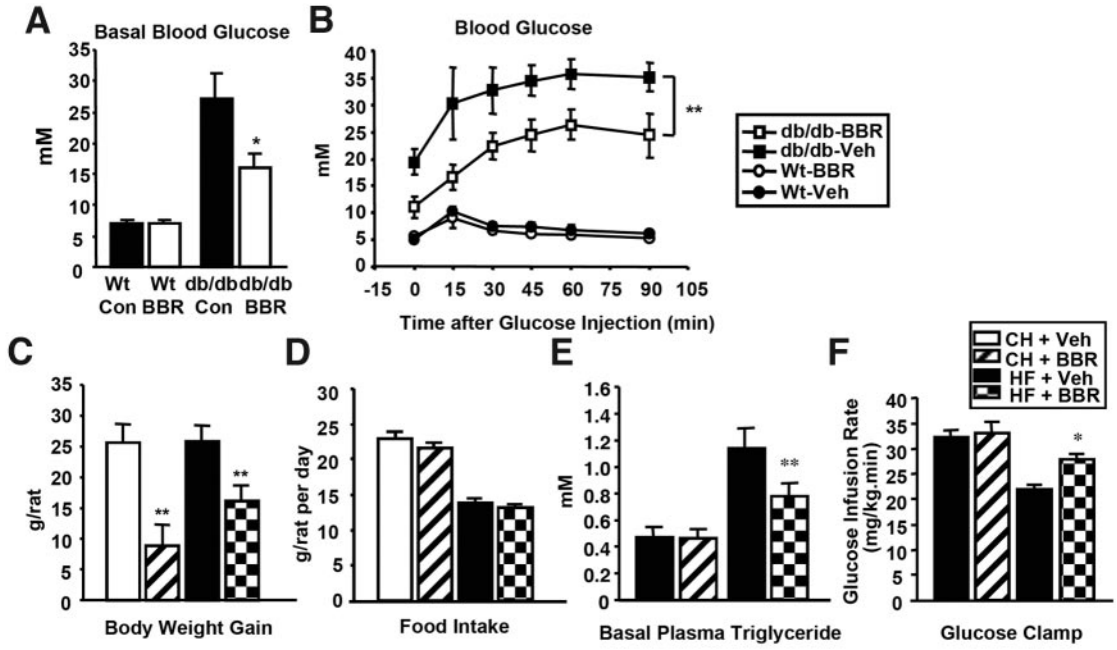


FIG. 3. Effects of berberine on in vivo metabolism in two animal models of insulin resistance. Wild-type and *db/db* mice were treated with berberine, and basal blood glucose (*A*) and glucose tolerance (*B*) were measured. *db/db* mice spontaneously develop basal hyperglycemia as shown in the control (*db/db*-Con) versus wild-type groups. *A*: Basal hyperglycemia was significantly reduced in *db/db* mice gavaged with berberine (BBR; 560 mg · kg^{-1} · day^{-1}) for 7 days, compared with controls (vehicle-only gavage). *B*: Glucose tolerance test. Intraperitoneal bolus glucose (1 g/kg) was given to wild-type (Wt) mice, and glucose loads to *db/db* mice were then matched to the lean wild-type littermates. *C–F*: Berberine was also administered to chow-fed and high-fat–fed rats followed by measurement of body weight gain (*C*), food intake (*D*), basal plasma triglyceride (*E*), and whole-body insulin sensitivity as determined by hyperinsulinemic euglycemic clamp (*F*). Rats were gavaged with vehicle (Veh) or berberine (380 mg · kg^{-1} · day^{-1}) for 2 weeks before (but not including) the acute clamp study. For clamp studies (insulin infusion 0.25 units · kg^{-1} · h^{-1}), jugular and carotid cannulae were implanted 7 days previously and animals were studied over 2 h in the conscious state after 5–7 h fasting. Group sizes were 4–5 mice for *A–B*, >10 rats for *C–E*, and >6 for *F*. *P < 0.05; **P < 0.01 vs. corresponding control group.

rats and mice for the measurement of plasma levels of cholesterol and triglycerides. Liver samples from rats were used for the measurement of triglyceride content as previously described (15).

Histological analysis and morphometry. The adipose tissues from the vehicle-treated controls or the berberine-treated *db/db* mice were embedded in a tissue-freezing medium (Leica) and frozen in liquid nitrogen immediately after decapitation and stored at −80°C. Tissue sections (50 μm) were prepared with a cryostat microtome and mounted on gelatin-coated glass slides. After paraformaldehyde fixation, they were stained with hematoxylin and eosin and photographed at ×100 magnification. The sectional areas of white adipose tissue (WAT) were analyzed for the purpose of quantifying the number and size of adipocytes.

Western blot analysis. Protein samples denatured in SDS sample buffer (125 mmol/l Tris-HCl, pH 6.8, 50% glycerol, 2% SDS, 5% β-mercaptoethanol, and 0.01% bromophenol blue) were subjected to SDS-PAGE and blotted onto polyvinylidene difluoride (Millipore) membranes. Blotted membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 for 1 h and then incubated with primary antibodies against AMPK (Cell Signaling Technology), phosphoAMPK (Thr^{172}) (Cell Signaling Technology), acetyl-CoA carboxylase (Upstate), and phosphoACC (Upstate) for 16 h at 4°C. After three washes in Tris-buffered saline containing 0.1% Tween 20, the membranes were incubated with anti-mouse or anti-rabbit IgG and horseradish peroxidase–linked antibodies for 2 h. Immunoreactive signals were detected with the WEST-1 Western blot detection system (Intron, Kyungki-Do, Korea) and quantified with a LuminoImager (LAS-3000) and Science Lab 2001 Image Gauge software (Fuji Photo Film).

Luciferase reporter assay. HEK293 cells were transfected 1 day before confluence by the calcium phosphate method described previously (20). All the transfection experiments were performed in duplicate and repeated independently at least three times.

Statistical comparisons. Data were compared using paired Student's *t* tests or ANOVA as appropriate and are presented as means ± SE.

RESULTS

Effects of berberine in *db/db* mice. To investigate the in vivo metabolic properties of berberine, we initially examined its effects when administered to *db/db* mice because these animals exhibit marked obesity as well as impaired glucose tolerance. Berberine was administered daily (5 mg · kg^{-1} · day^{-1}) intraperitoneally for 3 weeks to 12-week-old *db/db* mice. Body weight was significantly reduced in berberine-treated animals (Fig. 1*A*). Whereas the vehicle-treated mice showed normal body weight gain during the experimental period (Fig. 1*B*), berberine treatment resulted in a gradual loss (~13%) of body weight over the same period (Fig. 1*B*). Importantly, food intake was not significantly different between vehicle and berberine-treated animals. Consistent with this, the expression of several hypothalamic neuropeptides, known to be involved in regulating food intake, were not significantly affected by berberine treatment (Fig. 1*C–E*). Gross inspection of berberine-treated mice indicated a clear reduction in adipose tissue mass (Fig. 1*A*). Quantification of this effect revealed that epididymal fat mass was reduced by ~10% when normalized for body weight (Fig. 2*A* and *B*). Computer tomography revealed that both visceral and subcutaneous fat depots were similarly reduced by berberine (Fig. 2*C*). Histological studies indicated that the reduction in fat mass by berberine treatment was principally due to reduced adipocyte size (Fig. 2*D*). The average diameter of the white fat cells in control and berberine-treated *db/db* mice was 54.13 ± 9.74 and 31.96 ± 7.28 μm, respectively (Fig. 2*E* and *F*). These results suggest that berberine reduces fat mass primarily by decreasing the size of fat cells rather than fat cell number.

To assess glucose homeostasis and insulin sensitivity in *db/db* mice treated with berberine, we next performed glucose tolerance tests. Berberine administration resulted in a significant reduction in fasting blood glucose levels

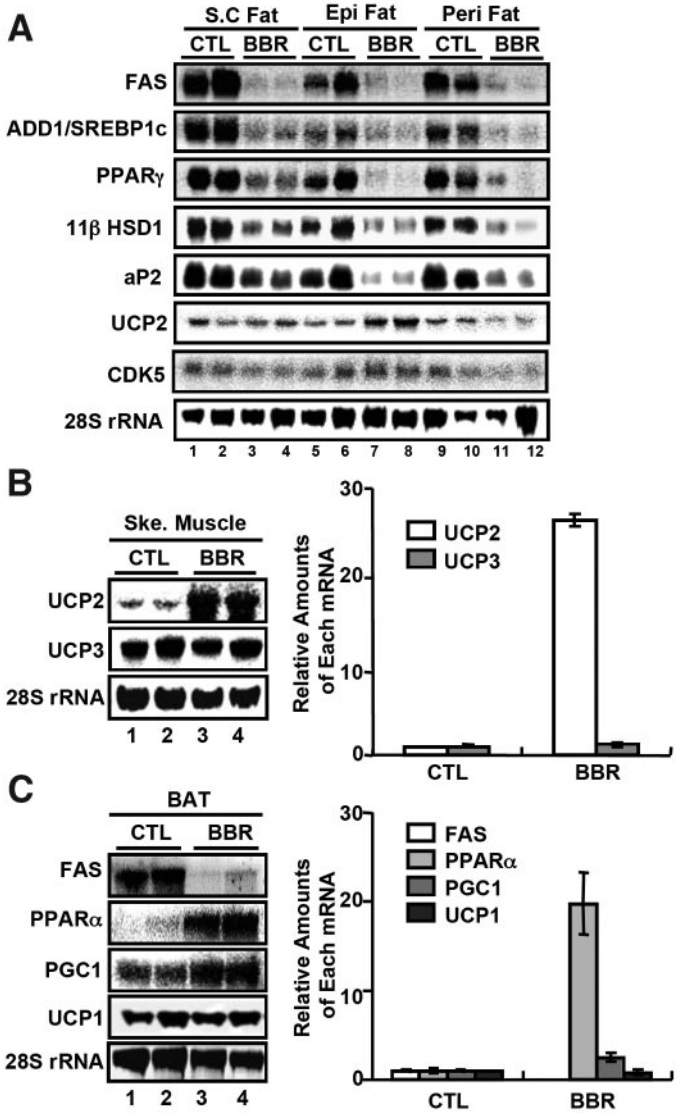


FIG. 4. Effects of berberine (BBR) on expression of metabolic genes in fat and muscle. *A*: Northern blot analysis of gene expression in subcutaneous (S.C Fat), epididymal (Epi Fat), and perirenal fat (Peri Fat) tissues. *B*: Northern blot analyses of UCP2 and UCP3 mRNA in skeletal muscle (quadricopes). *C*: Northern blot analyses of FAS, PPARα, and PPARγ coactivator-1 (PGC1) mRNA in brown adipose tissue.

combined with a significant improvement in glucose tolerance (Fig. 3). There was no significant effect on basal glucose or glucose tolerance in normal wild-type mice.

Effects of berberine in high-fat–fed rats. Another animal model that is frequently used to study insulin resistance is the high-fat–fed rat. Berberine was administered orally to chow-fed and high-fat–fed rats for 2 weeks. Consistent with the data in *db/db* mice, we observed a significant reduction in body weight gain following berberine treatment (Fig. 3). This reduction in body weight was observed in both chow- and high-fat–fed animals, without a concomitant change in food intake (Fig. 3*C* and *D*). Importantly, berberine was well tolerated in the animals, as indicated by the fact that food intake was unaltered by the drug, and necropsy and histological analysis of major organs such as the liver and kidney revealed no adverse pathology or inflammation (data not shown).

We next performed hyperinsulinemic-euglycemic clamp studies in control and high-fat–fed rats administered either vehicle or berberine (380 mg/day) by daily gavage for 2 weeks. Plasma triglycerides, measured in the basal state before the clamp, were significantly lowered in the high-fat–fed group treated with berberine (Fig. 3*E*). The untreated high-fat–fed group had a significantly lower clamp glucose infusion rate compared with the chow-fed group, demonstrating whole-body insulin resistance (Fig. 3*F*). Berberine treatment significantly lessened this insulin resistance in high-fat–fed rats, as indicated by an increased clamp glucose infusion rate (Fig. 3*F*). Plasma triglyceride and clamp glucose infusion rate were not altered by berberine treatment in normal chow-fed rats (Fig. 3*E* and *F*). We have also obtained preliminary data to show that insulin stimulated suppression of hepatic glucose output was enhanced following 2 weeks of treatment with berberine in rats (data not shown).

Berberine alters the expression of metabolic genes in fat and muscle in vivo. To investigate the mechanism of berberine action on insulin action and body weight, we next assessed the effects of berberine on the expression of certain genes that are known to play a critical role in energy balance. As shown in Fig. 4*A*, the expression of a number of adipocyte-specific genes including fatty acid synthase (FAS), ADD1/SREBP1c (adipocyte determination and differentiation–dependent factor 1/sterol regulatory element–binding protein 1c), peroxisome proliferator–activated receptor (PPAR)γ, 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), and aP2 was reduced in various WAT depots of berberine-treated mice. In contrast, the level of cyclin D kinase 5 mRNA was not significantly altered, indicating that the inhibitory effect of berberine on gene expression was restricted to adipogenic and lipogenic genes. Conversely, in skeletal muscle, the expression of uncoupling protein (UCP) 2 mRNA was significantly increased by berberine, while that of UCP3 was unchanged (Fig. 4*B*). In brown adipose tissue, the expression of PPARα mRNA also increased dramatically, while the mRNA of lipogenic genes such as FAS was reduced as in the case of WAT (Fig. 4*C*). Additionally, expression of PPARγ coactivator-1, a key regulator of several mitochondrial genes involved in adaptive thermogenesis, was substantially increased (Fig. 4*C*).

To further investigate the effects of berberine on overall gene expression, we compared genome-wide expression profiles in the WAT of berberine-treated mice and control mice using DNA microarrays. The genes that responded reproducibly to berberine were categorized by their fold induction (>1.5-fold, 996 genes) or repression (<0.8-fold, 1,483 genes) (online appendix Table 1 [available at http://diabetes.diabetesjournals.org]). Most genes involved in lipogenesis were downregulated by berberine treatment; for example, FAS and fatty acid desaturase 3, which control the final step of triglyceride synthesis from malonyl-CoA to palmitate and from phosphatidic acid to triglycerides, respectively, decreased 0.59- and 0.27-fold, respectively. Interestingly, 11β-HSD1, a key enzyme linked to visceral obesity and metabolic syndrome, decreased 0.63-fold, and expression of most genes involved in carbohydrate metabolism was also reduced (online appendix Table 1). In contrast, the transcript level of enzymes related to energy dissipation, including glycerol kinase and acyl-CoA dehydrogenase, increased 4.0- and 2.2-fold, respectively. These results imply that berberine treatment in vivo results in an altered gene expression profile that

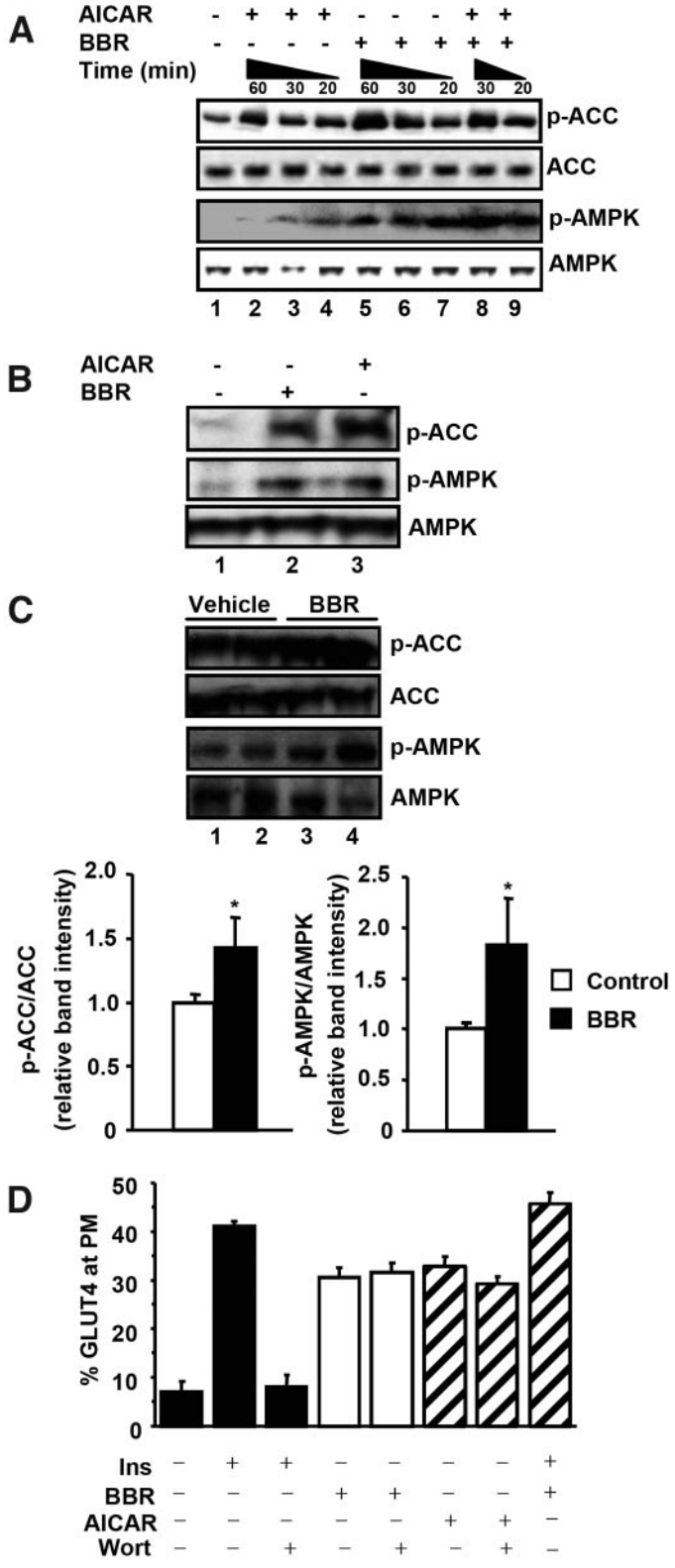


FIG. 5. Activation of AMPK and GLUT4 translocation by berberine. *A*: 3T3-L1 adipocytes were treated with DMSO, berberine (BBR; 5 μg/ml), or AICAR (0.5 mmol/l) after 16 h serum starvation with DMEM supplemented with 0.1% BSA for different time periods as indicated. AICAR was used as a positive control for activating AMPK. *B*: L6 myotubes were incubated under basal conditions or in the presence of berberine (BBR) or AICAR for 30 min. Total cell lysates were subjected to Western blot analysis using antibodies specific for phospho-AMPK, phosphoACC, and total AMPK. Representative blots are shown. *C*: AMPK activation by administration of berberine (BBR) in vivo. Berberine or vehicle (controls) was injected intraperitoneally (5 mg/kg) into 9-week-old *db/db* obese and diabetic mice every day for 3 weeks. Livers were removed, lysed, and immunoblotted with antibodies specific for phosphoAMPK, phosphoACC, total AMPK, and total ACC. Quantitative data represents the means ± SE for $n = 6$ in each group. *D*: L6 myotubes were incubated under basal conditions or in the presence of insulin for 15 min, berberine (BBR) for 30 min, or AICAR for 30 min. Wortmannin (100 nmol/l) was added 5 min before the addition of the different agonists. The cell surface levels of the HA-GLUT4 reporter were measured in myotubes using a fluorescence assay, and data are expressed as the amount of HA-GLUT4 at the surface as a percent of the total expression. Data shown are the means ± SE of four to five separate experiments.

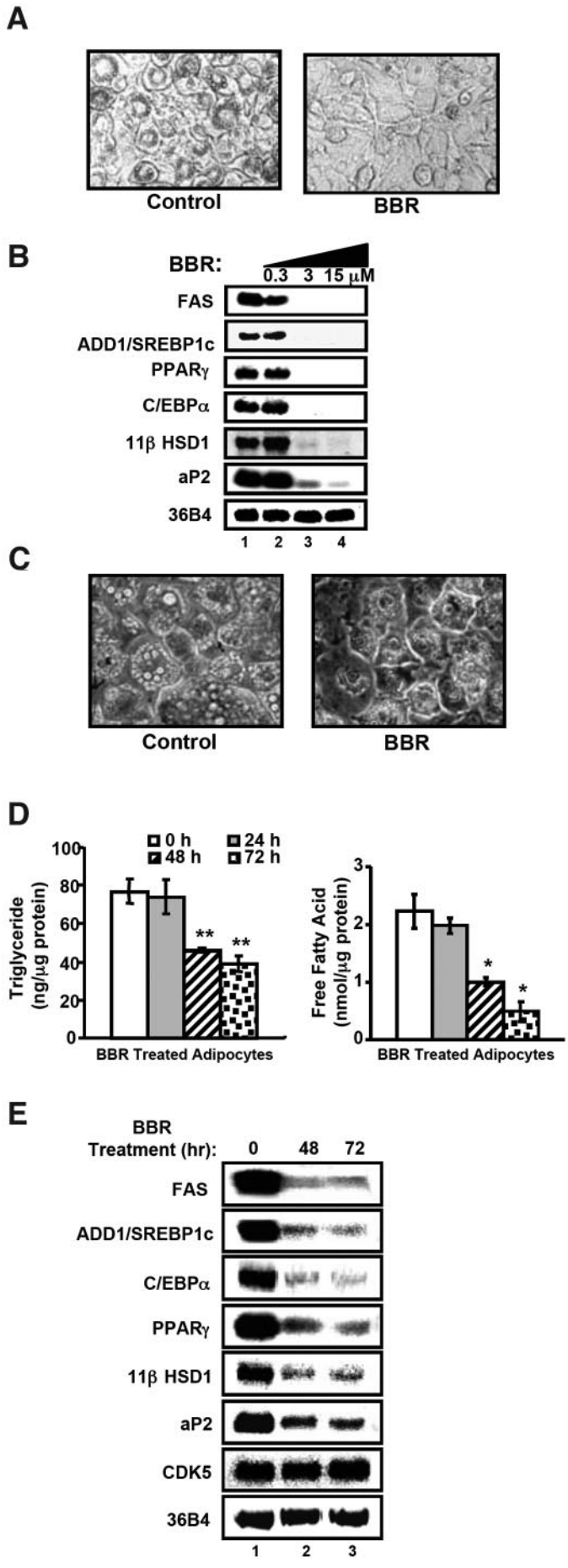
would promote catabolism of high energy intermediates (online appendix Fig. 1).

Berberine activates AMPK in adipocytes, myotubes, and liver. AMPK has previously been shown to play a key role as an energy sensor in metabolic tissues by coordinating both short- and long-term metabolic changes that lead to improved energy production and reduced energy storage (21). In view of the changes in gene expression observed with berberine treatment (Fig. 4), we hypothesized that this might be facilitated via activation of AMPK. To determine whether the effects of berberine that we have observed in animals could be mediated by activation of AMPK, we examined the effects of berberine on AMPK phosphorylation in adipocytes and myoblasts in vitro, since this has been shown to correlate with kinase activity. Furthermore, we also examined phosphorylation of ACC, as this is a major substrate of AMPK (22–24). Intriguingly, the effects of berberine on AMPK activity in adipocytes were more pronounced than that of 5-aminoimidazole-4-carboxamide riboside (AICAR), a known AMPK agonist (Fig. 5*A*). As shown in Fig. 5*A–C*, AMPK phosphorylation and ACC phosphorylation were increased in myoblasts and adipocytes in vitro after short-term treatment with berberine and in liver after long-term berberine treatment of *db/db* mice.

Berberine stimulates GLUT4 translocation in L6 myotubes. One of the major acute actions of AMPK is to stimulate GLUT4 translocation in muscle (25). As shown in Fig. 5*D*, insulin and the AMPK agonist AICAR increased the cell surface levels of GLUT4 by four- and threefold, respectively. In agreement with previous studies (26,27), the phosphatidylinositol (PI) 3′ kinase inhibitor wortmannin blocked insulin-stimulated GLUT4 translocation but had no effect on AICAR stimulation (Fig. 5*D*). Strikingly, berberine also stimulated GLUT4 translocation in L6 myotubes by more than threefold, and, like AICAR, this effect was unaffected by wortmannin (Fig. 5*D*). Consistently, we could not observe additive effects of berberine and insulin on GLUT4 translocation over periods of ≥2 h exposure to berberine (Fig. 5*D*).

Effects of berberine on adipocytes. AMPK activation has been shown to lead to lipid lowering consistent with our in vivo data (28) (Fig. 3*E*). To further test this in vitro, we next treated 3T3-L1 cells with or without berberine during adipocyte differentiation and found that triglyceride accumulation was strongly inhibited (Fig. 6). Vehicle-treated adipocytes displayed normal differentiation, as indicated by the appearance of numerous intracellular lipid droplets. However, berberine caused a dramatic reduction in lipid droplet accumulation and adipogenic gene expression (Fig. 6*A* and *B*), implying that berberine would indeed attenuate adipocyte differentiation.

We next investigated the effects of berberine on lipid metabolism in fully differentiated adipocytes. Berberine-



treated 3T3-L1 adipocytes had significantly reduced levels of intracellular triglycerides and free fatty acids compared with vehicle-treated cells (Fig. 6*C* and *D*). To determine the molecular mechanism for the lipid-lowering properties of berberine in 3T3-L1 adipocytes, we measured the transcript levels of a number of adipocyte-specific genes. Consistent with the in vivo gene expression data (Fig. 4), the levels of most lipogenic transcripts, including FAS, ADD1/SREBP1c, PPARγ, C/EBPα, 11β-HSD1, and aP2, were decreased by berberine (Fig. 6*E*), whereas the expression of nonadipogenic genes, such as cyclin D kinase 5, was not affected. Berberine did not cause a significant loss of cell viability or affect the growth rate of NIH-3T3 fibroblasts (online appendix Fig. 2). Furthermore, we observed that berberine (15 μmol/l) significantly reduced GLUT4 mRNA expression as well as several lipogenic and adipogenic genes in 3T3-L1 adipocytes, suggesting that chronic administration of berberine could provoke dedifferentiation of adipocytes in vitro (online appendix Fig. 3).

Berberine stimulates phosphorylation of p38 and PPARγ. Many of the genes that undergo a reduction in expression in response to berberine treatment are PPARγ-responsive genes. Thus, it is possible that berberine may inhibit the activity of PPARγ. It has previously been described that PPARγ transcriptional activity is regulated by phosphorylation and that the upstream kinase for this effect is p38 mitogen-activated protein kinase (MAPK) (29–34). We also observed that phosphorylation of both p38 MAPK and PPARγ were increased by berberine in adipocytes (Fig. 7*A*). To confirm that berberine inhibits PPARγ activity, we next examined its effects in luciferase reporter assays. These studies demonstrated that berberine inhibited the transcriptional activity of PPARγ in a dose-dependent manner (Fig. 7*B*). The effect of berberine to inhibit PPARγ activity was quantitatively similar to that observed with bisphenal A diglycidyl ether, a known PPARγ antagonist (35).

DISCUSSION

Berberine has been used as a therapeutic to treat a variety of human diseases in Korea, China, and possibly other Asian countries that practice the use of traditional medicines. Although its most common use is in the treatment of diarrhea and as an antitumor and antimicrobial agent (36–39), there are some reports of its potential use for the treatment of human diabetes (13,40,41). In one clinical study, 60 patients with type 2 diabetes were treated with berberine for 1–3 months, and 90% of patients showed improvement in their clinical symptoms (11). Very recently, Kong et al. (42) described the cholesterol-lowering properties of berberine. In the present study, we have extended these previous investigations and provide evidence that berberine reduces body weight and lipid levels

FIG. 6. Effects of berberine on adipogenic gene expression and lipid metabolites. *A*: Microscopic views of 3T3-L1 cells. 3T3-L1 cells were differentiated into adipocytes in the absence or presence of berberine (BBR; 3 μmol/l). *B*: Northern blot analysis. Total RNA was isolated from 3T3-L1 adipocytes treated with DMSO (*lane 1*) or berberine (*lane 2*, 0.3 μmol/l; *lane 3*, 3 μm; and *lane 4*, 15 μmol/l) for 9 days. Blots were hybridized with adipocyte-specific gene probes. Expression of 36B4 was used as a loading control. *C*: Microscopic views of differentiated adipocytes treated without or with berberine (BBR; 15 μmol/l) for 72 h. *D*: Intracellular lipid content of adipocytes in the absence or presence of berberine (BBR; 15 μmol/l). *E*: Alteration of adipocyte gene expression by berberine (BBR). Total RNA was isolated from 3T3-L1 adipocytes treated with DMSO (*lane 1*) and berberine (15 μmol/l; *lane 2* for 48 h and *lane 3* for 72 h). Northern blots were analyzed with cDNA probes.

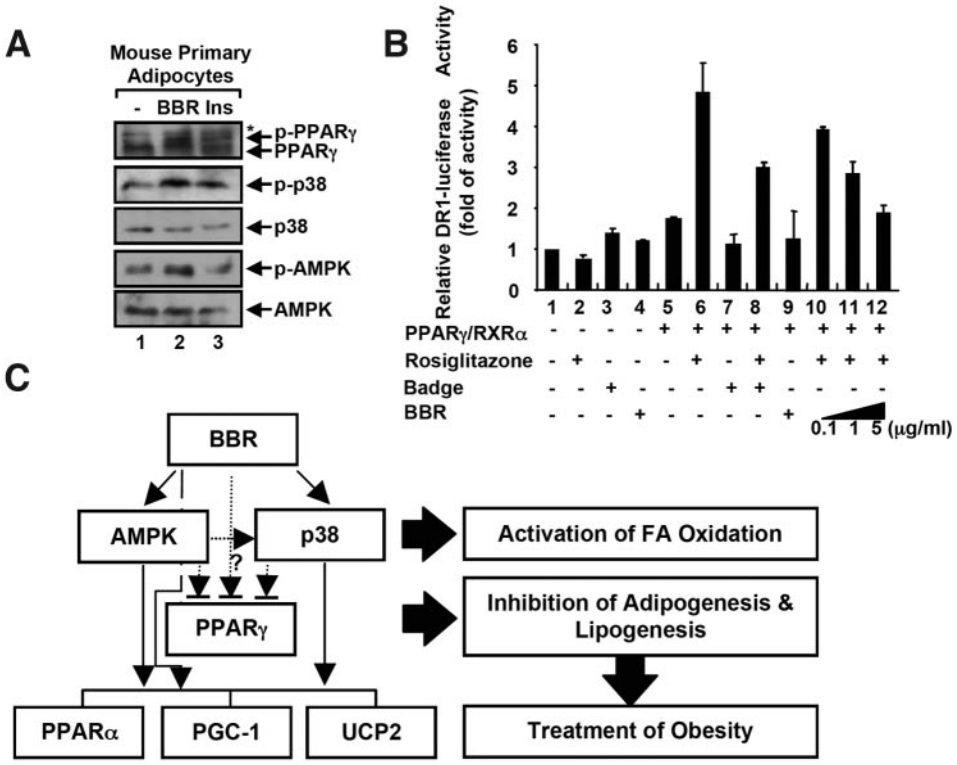


FIG. 7. Activation of AMPK and p38 MAPK and inhibition of PPARγ activity in adipocytes by berberine. *A*: Western blot analysis of AMPK, p38 MAPK, and PPARγ phosphorylation by berberine (BBR). Mouse primary adipocytes were isolated and treated with DMSO, berberine, or insulin for 20 min in DMEM supplemented with 0.1% BSA. *B*: h293 cells were transfected with DR-1-luciferase reporter construct along with PPARγ and RXRα expression vectors, as indicated. After 12 h serum starvation with DMEM media supplemented with 0.1% BSA, cells were treated with DMSO, rosiglitazone (100 nmol/l), bisphenol A diglycidyl ether (50 μmol/l), or berberine for an additional 12 h. *C*: Schematic model of berberine (BBR) in control of energy metabolism. Berberine stimulates fatty acid oxidation by activating AMPK and p38 and increasing PPARα, UCP2, and PPARγ coactivator-1 (PGC-1) in peripheral tissues. It also inhibits adipogenesis by suppressing the transcriptional activity PPARγ by increasing inhibitory phosphorylation of PPARγ.

and improves insulin action in two separate animal models of insulin resistance. Most importantly, we show that berberine acutely activates AMPK activity in both adipocytes and myocytes, and within these cell types berberine induces a variety of metabolic effects consistent with AMPK activation. These include activation of GLUT4 translocation; increased phosphorylation of AMPK, ACC, and p38 MAPK; reduced lipid content in adipocytes; increased expression of genes involved in lipid oxidation; and decreased expression of genes involved in lipid synthesis.

The present data indicate that AMPK is a major intermediate in facilitating the beneficial effects of berberine. These effects are likely manifest both as short- and long-term effects. The acute effects probably involve increased GLUT4 translocation in muscle cells and increased trafficking of free fatty acids into mitochondria via increased ACC phosphorylation, both of which contribute to glucose and lipid lowering. We could not observe acute effects of berberine on blood glucose, while AICAR was successful to reduce blood glucose acutely. However, acute administration of other AMPK agonists, such as metformin, also had no effect on blood glucose, suggesting that blood glucose–lowering action is not essential for AMPK agonist. The chronic effects involve changes in gene expression, which likely contribute to reduced fat cell differentiation and increased mitochondrial biogenesis, again contributing to lipid lowering, reduced fat mass, and improved insulin sensitivity. We have observed a significant reduction in the expression of lipogenic genes in adipose tissue following treatment with berberine either in vitro or in vivo. Moreover, our in vitro studies in 3T3-L1 cells showed that berberine inhibited adipocyte differentiation probably by inhibiting PPARγ activity. It is now well established that PPARγ is an important transcriptional regulator of adipogenesis (43,44). In addition, the activity of PPARγ can be regulated by a variety of mechanisms, including phosphorylation by members of the MAPK family (29–31,33,34). In particular, p38 MAPK has been shown to enhance PPARγ phosphorylation, which leads to inhibition of its transcriptional activity, resulting in a blockade in fat cell differentiation (29,31). AMPK has been implicated as an upstream regulator of p38 MAPK (45), thus providing a link between the activation of AMPK and fat cell differentiation and control of lipogenic gene expression. Our data are consistent with such a mechanism because we have observed that berberine treatment increases the phosphorylation of AMPK, p38, and PPARγ in adipocytes, suggesting that this may be a major regulatory pathway by which this compound mediates its actions. It is also conceivable that this pathway plays an important role in lipogenesis in other tissues such as the liver because we also observed increased phosphorylation of AMPK and p38 in berberine-treated FAO rat hepatoma cells (W.S.K., Y.S.L., J.B.K., unpublished data). A further prediction from our work is that berberine leads to increased whole-body energy expenditure. Indeed, we observed that berberine-treated animals exhibited increased oxygen consumption and core body temperature (data not shown) with concurrent increase in the expression of genes involved in the control of energy expenditure, such as UCP2 (46,47). Similar metabolic adaptations to those described above have been ascribed to AMPK in other contexts (48,49), again supporting the conclusion that berberine mediates many of its metabolic actions via AMPK.

It is intriguing to compare the metabolic effects of berberine with other insulin-sensitizing agents such as the TZDs or metformin. Based on the data presented here, berberine and metformin share a number of features in common. Metformin causes weight reduction, improved insulin sensitivity, and lipid lowering in both human and animal models of insulin resistance (50). A major mode of action of metformin is activation of AMPK, particularly in

liver (51), although it has also been shown to stimulate AMPK activity in adipose tissue (52). The latter effect is particularly germane to the present studies because one of the major disadvantages of TZDs is that while they lead to improved insulin sensitivity and lipid lowering, they also lead to increased adiposity due to their stimulatory effects on adipocyte differentiation (53). In contrast, berberine and metformin have the opposite effects on adiposity. This may reflect the fact that berberine and metformin have a direct effect on AMPK activity in a variety of tissues, whereas TZDs principally activate AMPK in the liver indirectly via increased adiponectin secretion, while at the same time sequestering fatty acids in adipose tissue via the "lipid steal" effect (54).

Collectively, these data are consistent with a model whereby berberine activates AMPK in multiple tissues, including adipose tissue and muscle (Fig. 7*C*). This increase blocks adipose tissue differentiation presumably via p38 MAPK–mediated phosphorylation of PPARγ and increases free fatty acid oxidation either directly by reducing ACC activity, thus allowing for increased trafficking of nutrients into the mitochondria, or indirectly via upregulation of genes that regulate energy expenditure. A major question arising from these studies concerns the mechanism by which berberine activates AMPK. It is highly unlikely that these effects are mediated indirectly via increased secretion of some cellular factor because we have observed rapid activation of AMPK in several cell lines in vitro. One possibility is that berberine directly effects the integrity of the mitochondria, perhaps via a similar mechanism to metformin, and this mechanism is currently under investigation.

In conclusion, berberine, a purified component from a traditional oriental medicine, reduces whole-body adiposity and improves insulin sensitivity in two separate animal models of insulin resistance, at least in part, by activating AMPK in multiple cell types. Collectively, these effects lead to changes in biochemical processes and gene expression that lead to a net switch in the metabolic program of the organism to catabolism of fuel stores, an adaptation that may be of some benefit in the face of disorders characterized by insulin resistance.

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