J Food Sci Nutr
Vol 14, p 298~302 (2009)
DOI: 10.3746/jfn.2009.14.4.298

J Food Science and Nutrition

Anti-obesity Effect of Berberine in Mice Fed a High Fat Diet

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Abstract

We investigated the anti-obesity effect of berberine in mice fed a high fat diet and focused on the analysis of adipogenesis in epdidymal adipose tissue. Male C57BL/6J mice were divided into three groups, which were fed either a normal diet (Nor), a high fat diet (HFD), or a high fat diet plus orally administered berberine (0.2 g /kg body weight) (HFD+B) for 8 weeks. Relative to mice in the HFD group, mice in the HFD+B group showed significant reductions in weight gain and adipose tissue weight. Serum triglyceride levels in mice from the HFD+B group were significantly lower than those of the HFD mice, as were the levels of serum insulin and leptin. An effect of berberine to reduce epididymal adipose mass was revealed by H&E staining. Berberine inhibited the high fat diet-induced increase in levels of the proteins CD36 and CCAAT/enhancer-binding protein α (C/EBPα) observed in epididymal adipose tissues of mice from the HFD group. These results suggest that berberine has an anti-obesity effect in mice and that the effect is mediated by inhibition of adipogenesis.

Key words: anti-obesity, berberine, adipose tissue, C/EBPα

INTRODUCTION

The World Health Organization (WHO) designated obesity as the one of the 10 leading contributors to mortality globally. It is reported that approximately half a million people in the USA and Western Europe die as a result of obesity-related diseases (1). A significant portion of the Korean population (32.4%) also meets the criteria for obesity (2). Obesity is mainly caused by an imbalance between the intake and expenditure of energy. A prolonged obese state is implicated in a variety of disease such as diabetes, cardiovascular disease, and even certain cancers (3,4). In addition to enlarged adipose tissues, obesity is characterized by high levels of triglycerides and pro-inflammatory mediators, as well as elevated glucose levels associated with insulin resistance (5,6). Adipose tissue plays important biological roles, such as the intracellular storage of lipids and the secretion of adipokines to regulate metabolism and the immune system (7). Hyperglycemia promotes adipogenesis and increases the expression of numerous proteins, such as peroxisome proliferator-activated receptor γ (PPARγ) isoforms and CCAAT/enhancer-binding protein α (C/EBPα), involved in glucose and lipid metabolism, insulin signaling, and cell differentiation (8).

If diet regulation and exercise are insufficient to reverse obesity, anti-obesity drugs may be of therapeutic value in some patients. Some medications, however, exhibit limited efficacy and may be accompanied by adverse effects such as high blood pressure, palpitations, and glaucoma (9). Therefore, developing safer and more effective anti-obesity treatments is a high priority.

Berberine is a natural product found in *Hydrastis Canadensis*, *Berberis,* and *Cortex phellodendri* (10). Traditionally, berberine has been used for its antimicrobial properties (11). Recent research has established that berberine activates AMP-activated protein kinase (AMPK) in diabetic mice. It also has been shown to attenuate hyperglycemia and insulin resistance in rats (12). Berberine was shown *in vitro* to inhibit adipocyte differentiation through the PPARγ pathway and to decrease the expression of adipogenic enzymes and inflammatory molecules in 3T3-L1 cell (13,14). Although berberine was demonstrated to have anti-adipogenic effects *in vitro*, its overall performance *in vivo* was poor. Therefore, in this study, we have evaluated the anti-obesity effects of berberine in mice fed a high fat diet, with a specific focus on adipogenesis.

MATERIALS AND METHODS

Chemicals

Berberine was purchased from Sigma Aldrich (St. Louis, MO, USA). A high fat diet formulation (34.9% fat) was purchased from Research Diets (NJ, USA). All

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other chemicals were of analytical grade or purer.

Animals and diets

Male C57BL/6J mice, 4 weeks of age, were purchased from Orient Bio Inc. (Gyeonggi, Korea) and were allowed free access to commercial chow for 7 days. After acclimation, the mice were divided randomly into three groups and maintained for 8 weeks on either a normal diet (Nor, n=10), high-fat diet (HFD, n=10), or high-fat diet + berberine (HFD + B, n=10). The mice received a single daily oral dose of 0.1% hydroxypropyl methylcellulose vehicle (HPMC; Nor and HFD groups) or berberine (0.2 g/kg body weight; HFD + B group). The dose of berberine was established as dose of 0.2 g/kg body weight according to a previous study that reported that berberine did not exhibit any toxicity in this level (12). The composition of the normal mouse diet has been described in our previous study (15). Food and water were provided *ad libitum*. Mice were maintained at 21～25°C with a humidity of 50～60% under a 12-hr:12-hr light : dark cycle (lights on at 06:30 hr). All animal procedures were conducted in accordance with the Guidelines for Institutional Animal Care and Use Committee of the Korea Food Research Institute.

Sample preparation

At the end of the 8-week experimental feeding period, the mice were fasted for 12 hr. Blood from the abdominal aorta was collected in a tube and centrifuged at $1500 \times g$ for 20 min to separate the serum, which was stored at -70°C until analysis. Epididymal and perirenal adipose tissues were removed, weighed, and stored at -70°C until use.

Serum analysis

Triglyceride (TG) levels were measured using a commercial enzyme kit (Eiken, Japan). Leptin was analyzed using a mouse leptin imuunoassay (R&D Systems, USA). An ELISA kit (Shibayaki, Japan) was used for the analysis of insulin.

Histological analysis of adipose tissue

A single piece of epididymal adipose tissue was removed from an identical location in each mouse. This was fixed in 4% formaldehyde in phosphate-buffered solution, embedded in paraffin, sliced, and stained with hematoxylin and eosin (H&E; Sigma-Aldrich, USA). The epididymal adipose tissues were observed under a microscope (Olympus IX71, Japan) and photographed with a digital camera (Olympus DP71, Japan). Cellular size was measured using Image J software (NIH, USA).

Western blot analysis

Epididymal adipose tissue was homogenized in PROPREP (Invitron, Korea) and centrifuged, and supernatant protein concentrations were measured using a Bradford assay (Bio-Rad, USA). Quantified proteins were separated by 6 or 10% SDS-PAGE polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (Millipore, USA). After transferring, the membranes were washed in TBST (10 mM Tris-HCl, 0.1% Tween, 150 mM NaCl, pH 7.6) and blocked with 5% skim milk in TBST. Western blots were then incubated with primary antibodies anti-CD36, anti-C/EBPα, and anti-B-actin overnight at 4°C, and then washed with TBST. Blots were incubated with horseradish peroxidase-linked secondary antibody for 2 hr, washed with TBST, and developed by enhanced chemiluminescence.

Statistical analysis

Data were expressed as mean ± SEM. All statistical analyses were carried out with ANOVA and Duncan's multiple-range test using SAS (Cary, NC, USA), with a value of $p<0.05$ selected as the cut off for statistical significance.

RESULTS AND DISCUSSION

Body weight, adipose tissue weight, and histology of adipose tissue

We evaluated whether berberine has an anti-obesity effect in mice fed a high fat diet for 8 weeks. Fig. 1 shows the effect of berberine on the body weight changes of experimental mice. At the end of 8 weeks, the mean body weight of mice in the HFD group was increased by approximately 54% compared to mice in the Nor group. However, the mean body weight HFD +

(g)

Fig. 1. Changes in body weight. Data are mean ± SEM values for 10 mice per group. Values at a time not sharing a common superscript letter are significantly different at $p<0.05$, as assessed using Duncan's multiple-range test. Nor, normal diet group; HFD, high-fat diet; HFD + B, high-fat diet mice treated orally with berberine (0.2 g/kg per day).

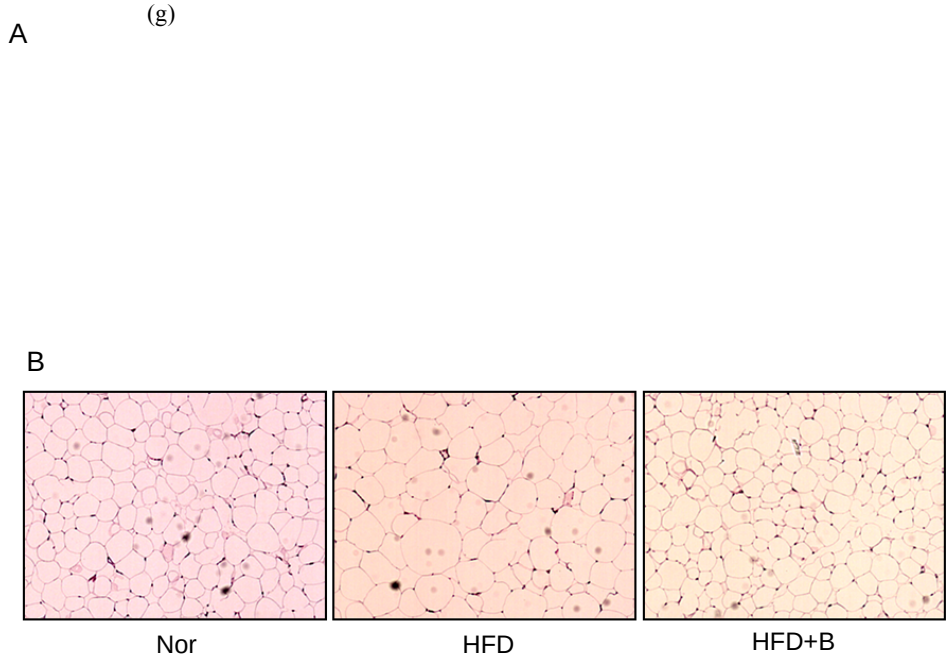

Fig. 2. Adipose tissues weights (A) and representative H&E images of epididymal adipose tissues (B). Data in A are mean±SEM values for 10 mice per group. Nor, normal diet group; HFD, high-fat diet; HFD+B, high-fat diet mice treated orally with berberine (0.2 g/kg per day).

B mice was significantly lower (about 37%) than their HFD counterparts. No significant differences in daily food consumption were found among the three groups: Nor: 2.94±0.54 g; HFD: 2.28±0.17 g; and HFD+B: 2.32±0.09 g. The effects of berberine treatment on adipose tissue weight and adipose cell size are shown in Fig. 2. The weights of epididymal and perirenal adipose tissue in the HFD group were increased over 200% compared to that of the Nor group. Adipose tissue weights from mice in the HFD+B group, however, were significantly lower than those from untreated HFD mice (Fig. 2A). This result is in agreement with previous studies showing berberine administration to produce a dose-dependent reduction of body weight in diabetic hyperlipidemic rats (16). Obesity is characterized by an excess of body fat (4), and adipocytes are ultimately enlarged by obesity. As shown in Fig. 2B, adipocytes in tissues from mice in the HFD group appear to be dramatically larger than those in tissues from mice in the Nor group. Adipocytes from mice in the HFD+B group appear to be much smaller than those from HFD mice, and similar in size to adipocytes from mice in the Nor group. Taken together, these results clearly demonstrate that berberine has an anti-obesity effect in high fat diet-induced obese mice.

Serum TG, leptin, and insulin

An elevated serum TG level is a major marker of obesity, together with elevated glucose, high blood pressure, and reduced high-density lipoprotein. The adipocyte is an inert tissue functioning as an energy store for TG and cholesterol esters. It also secretes various adipokines, such as leptin, adiponectin, and resistin, which regulate pathological processes. Leptin primarily controls appetite; however, the overflow of leptin leads to insulin resistance and the production of pro-inflammatory cytokines (7). Therefore, we investigated the effects of berberine on the serum levels of TG, leptin and insulin in diet-induced obese mice. As shown in Table 1, the mice in the HFD group showed a significant increase in the levels of serum TG and leptin compared to mice in the Nor group. However, the levels of serum TG and leptin in mice from the HFD+B group were reduced significantly relative to the levels in HFD mice. These findings are similar to those of a previous report that found that berberine decreased plasma TG levels in Wistar rats placed on a high-fat diet (12). Choi et al. (14) also reported that berberine reduced level of leptin in 3T3-L1 cells. Hyperinsulinemia is associated with obesity, metabolic syndrome, and insulin resistance (17).

Table 1. Effect of berberine on serum TG, insulin and leptin levels in experimental mice

	Nor	HFD	HFD+B
TG (mg/dL)	66.29±2.89^{c}	108.88±3.78^{a}	81.23±7.62^{b}
Insulin (ng/mL)	4.96±0.59^{b}	16.95±0.73^{a}	7.11±0.37^{b}
Leptin (ng/mL)	0.58±0.04^{b}	1.37±0.21^{a}	0.63±0.08^{b}

Data are mean±SEM values for 10 mice per group. Values in the same row not sharing a common superscript letter are significantly different at $p<0.05$, as assessed using Duncan's multiple-range test. Nor, normal group; HFD, high-fat diet; HFD+B, high-fat diet mice treated orally with berberine (0.2 g/kg per day).

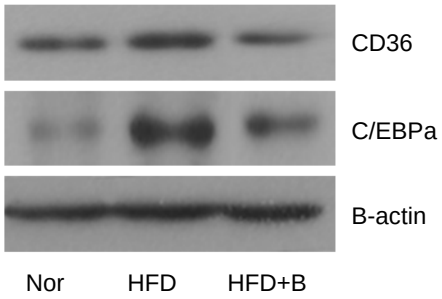

Fig. 3. Effect of berberine on protein expression in epididymal adipose tissue. Nor, normal group; HFD, high- fat diet; HFD + B, high-fat diet mice treated orally with berberine (0.2 g/kg per day).

Mice in the HFD group exhibited hyperinsulinemia, with a mean serum insulin level more than double that of mice in the Nor group. The serum insulin level in mice from the berberine-treated, HFD + B group was maintained at a level not different from that of mice in the Nor group. This result agrees with previous studies showing that berberine improved insulin action in high fat-fed Wistar rats (18,19). The levels of serum TG and leptin are associated with adipose cell size (7). In this study, the effect of berberine on serum TG and leptin levels is entirely consistent with the reduction in adipose cell size shown with H&E staining.

Changes of protein related adipogenesis in epididymal adipose tissue

To clarify the mechanism underlying the anti-obesity effect of berberine, we assessed the changes in adipogenesis-related protein expression in adipose tissue from mice using western blot analysis. Fig. 3 shows the effect of berberine on the levels of two proteins related to adipogenesis. The expression of CD36 in epididymal adipose tissue from mice in the HFD group was up-regulated relative to that in Nor mice. Berberine treatment apparently prevented the up-regulation of CD36 expression observed in the HFD group. It has been reported that CD36 participates in lipid metabolism in adipocytes (20). Yanfeng et al. reported that berberine inhibited CD36 expression by 24% *in vitro* (13). C/EBPα is transcription factor in adipogenesis, and therefore is an important factor for differentiation (21) and lipid metabolism (22) in adipocytes. As expected, berberine treatment clearly prevented the increase in expression of C/EBPα observed in the HFD group. Choi et al. reported that berberine inhibits C/EBPα expression in 3T3-L1 adipocytes (14). Therefore, it could be suggested that berberine inhibits adipogenesis through the regulation of CD36 and C/EBPα in adipose tissue.

In conclusion, this study clearly suggests that berberine has an anti-obesity effect in mice fed high fat diets. This beneficial effect of berberine may be mediated by the inhibition of adipogenesis.

ACKNOWLEDGEMENT

This study was supported by the Korea Food Research Institute and Platform Technology Project from Korea Industrial Technology Foundation.

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(Received November 24, 2009; Accepted December 3, 2009)