Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 60 (2011) 298 – 305
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Hypoglycemic and insulin-sensitizing effects of berberine in high-fat diet- and streptozotocin-induced diabetic rats

Yanwen Wang[a,b,*], Tony Campbell[a], Benjamin Perry[a,b], Cécile Beaurepaire[a], Ling Qin[a]

[a]Institute for Nutrisciences and Health, National Research Council Canada, Charlottetown, Prince Edward Island, Canada C1A 4P3
[b]Department of Biomedical Sciences, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada C1A 4P3

Received 21 September 2009; accepted 8 February 2010

Abstract

Hypoglycemic effects of berberine (BBR) have been reported in several studies in cell and animal models. However, the mechanisms of action are not fully understood. The present study was therefore aimed at determining the effect and underlying mechanisms of action of BBR on diabetes in a high-fat diet– and streptozotocin-induced diabetic rat model. Ninety male Sprague-Dawley rats, 150 to 170 g, were housed individually in cages. Two groups (n = 12 each) were fed the AIN-93G diet (normal control) and the same diet modified to contain 33% fat and 2% cholesterol (high-fat control), respectively. The third group (n = 66) was fed the high-fat diet and injected intraperitoneally 2 weeks later with 35 mg/kg body weight of streptozotocin in citrate buffer (pH 4.5). The rats in both control groups were injected with the vehicle. After 12 days, rats with semifasting (5 hours) blood glucose levels between 14 and 25 mmol/L were divided into 4 groups (n = 12 each) and treated with 0 (diabetic control), 50, 100, and 150 mg/kg/d of BBR for 6 weeks while continuing on the high-fat diet. Hypoglycemic effects of BBR were consistently demonstrated by semifasting and fasting blood glucose levels, and insulin-sensitizing effects were seen during oral glucose tolerance testing. Berberine also reduced food intake while having no effect on body weight in diabetic rats. No effect of BBR was observed on plasma levels of insulin, adipokines (leptin and adiponectin), or inflammatory cytokines (tumor necrosis factor–α and C-reactive protein). Berberine did not affect the state of oxidative stress as assessed by the activity of superoxide dismutase and the concentrations of malondialdehyde and reduced and oxidized glutathione in the liver. These findings demonstrated the hypoglycemic and insulin-sensitizing capabilities of BBR, with the underlying mechanisms awaiting further investigation.

1. Introduction

Type 2 diabetes mellitus (T2DM) prevalence has been increasing rapidly over the last decade and is now considered a worldwide epidemic. Although several drugs are available for the treatment of diabetes, adverse effects and drug resistance are of great concern. As an alternative, a greater number of people are seeking natural products or dietary interventions to prevent or treat diabetes. Several studies have shown beneficial effects of the plant alkaloid berberine (BBR) on diabetes. Except for its effect on insulin secretion, the antidiabetic effect of BBR has been linked to the inhibition of oxidative stress and chronic inflammation, and the regulation of lipid metabolism [1-3]. It is reported that BBR is capable of suppressing oxidative stress by increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD) and blocking malondialdehyde (MDA) formation [4,5], as well as attenuating glutathione (GSH) depletion [6]. Berberine has also been shown to have anti-inflammatory effects in animal models of acute and chronic inflammation [7]. Tumor necrosis factor–α (TNF-α) is an important proinflammatory cytokine produced mainly in macrophages, and it has been shown that BBR inhibits TNF-α gene expression in these cells [8].

The adipokines are signaling proteins involved in the regulation of energy and glucose metabolism [9,10]. Leptin regulates appetite and energy metabolism [10], and elevated levels in the blood indicate an increased risk for leptin resistance and T2DM development in middle-aged adults [11]. Adiponectin is involved in the regulation of glucose utilization and fatty acid catabolism [12], and decreased

* Corresponding author. Institute for Nutrisciences and Health, National Research Council Canada, Charlottetown, Canada PE C1A 4P3. Tel.: +1 902 566 7953; fax: +1 902 566 7468.
E-mail address: yanwen.wang@nrc.ca (Y. Wang).

0026-0495/$ – see front matter.
doi:10.1016/j.metabol.2010.02.005

blood levels are associated with insulin resistance [12,13]. Recent in vitro studies using 3T3-L1 adipocytes showed a significant reduction of leptin and increase of adiponectin after BBR treatment [14,15].

Impaired blood lipid levels are also characteristic of subjects with insulin resistance and T2DM [16], especially elevated circulating free fatty acids (FFAs) [17]. It is reported that FFAs are inversely related to pancreatic β-cell response to glucose [18], and elevated blood FFAs contribute to the development of insulin resistance and T2DM [19]. In a human study, it was found that a progressive increase in plasma FFAs caused a dose-dependent inhibition of insulin-stimulated glucose uptake and utilization [20]. Berberine reduces FFA levels in rats with impaired glucose tolerance [21], and a recent in vitro study showed that BBR can reverse FFA-induced insulin resistance in adipocytes [22].

Type 2 diabetes mellitus is a complicated metabolic disease characterized by impairment of both insulin secretion and insulin sensitivity [2]. Although the antidiabetic effects of BBR have been reported in a few studies [2,3,23-25], more evidence is needed to support its insulinotropic and insulin-sensitizing capabilities. In addition, the mechanisms remain largely uncertain [23], and data on the effect of BBR on insulin secretion, chronic inflammation, and circulating levels of FFA and adipokines are very limited, especially from in vivo studies. Therefore, the aim of the present study was to examine the insulinotropic, insulin-sensitizing, and hypoglycemic effects of BBR in a high-fat diet– and low-dose streptozotocin (STZ)–induced diabetes rat model. The effects of BBR on the plasma concentration of adipokines, inflammatory cytokines, liver biomarkers of oxidative stress, and blood lipids, particularly FFA, were also investigated.

2. Materials and methods

2.1. Animals and diets

Ninety male Sprague-Dawley rats (Charles River Laboratories, Montréal, Québec, Canada), 150 to 170 g, were housed individually in cages in a temperature-controlled room with a 12-hour light:dark cycle. After 1 week of acclimation with free access to regular rodent chow and water, the rats were randomly divided into 3 groups. Group 1 (n = 12, normal control [NC]) was fed a casein-cornstarch-sucrose–based semisynthetic AIN-93G diet containing 5% fat (beef tallow:sunflower oil mix [96:4, wt/wt]). Groups 2 (n = 12, high-fat control [HFC]) and 3 (n = 66) were both fed the AIN-93G diet modified to contain 33% fat and 2% cholesterol. After 2 weeks of high-fat diet feeding, group 3 was injected (intraperitoneally) with STZ (Sigma Chemicals, Oakville, Ontario, Canada) dissolved in citrate buffer (pH 4.5) at a dose of 35 mg/kg body weight and tested for semifasting (5 hours) blood glucose levels 3 and 12 days post-injection. The control animals were injected with the citrate buffer vehicle. Rats in group 3 with semifasting blood glucose levels between 14 and 25 mmol/L were randomly divided into 4 groups (n = 12 each) and continued on the high-fat diet. One group was used as a high-fat diabetic control (HFDC), and the other 3 were orally gavaged with BBR chloride (98% pure, Sigma Chemicals) dissolved in 0.5% carboxymethyl cellulose at doses of 50, 100, and 150 mg/kg body weight per day and designated as *BBR50*, *BBR100*, and *BBR150*, respectively. Rats in the NC, HFC, and HFDC groups were gavaged with 0.5% carboxymethyl cellulose. Body weights and food intake were recorded weekly and daily, respectively. Fasting blood glucose and oral glucose tolerance tests (OGTTs) were performed as described below.

At the end of the study, animals were fasted overnight and anesthetized with isoflurane (Pharmaceutical Partners of Canada Inc.). Blood samples obtained from the abdominal aorta were collected into EDTA tubes and placed on ice. After centrifugation, plasma was collected and stored at −80°C. Liver samples were obtained, immediately frozen in liquid nitrogen, and stored at −80°C. The animal use and experimental protocols were approved by the Joint Animal Care and Research Ethics Committee of the National Research Council Canada–Institute for Nutrisciences and Health and the University of Prince Edward Island. The study was conducted in accordance with the guidelines of the Canadian Council on Animal Care.

2.2. Fasting blood glucose measurements and OGTT

Throughout the 6-week treatment period, semifasting (5 hours) blood glucose was measured weekly on lateral tail vein blood samples using an ACCU-Check glucose meter (Roche Diagnostics, Toronto, Ontario, Canada). Oral glucose tolerance testing was also performed during the last week of treatment using a standardized method [16]. After a 12-hour fasting, the animals were orally gavaged with 2 g/kg body weight of glucose dissolved in water (40%, wt/vol). Blood glucose levels were measured at 0, 15, 30, 60, and 120 minutes with the glucose meter.

2.3. Plasma insulin, leptin, and adiponectin measurements

Plasma insulin, leptin, and adiponectin levels were quantified in duplicate using commercial enzyme-linked immunosorbent assay kits purchased from Crystal Chem (Downer's Grove, IL). Each assay was performed following the kit instructions. Standards at a series of concentrations were run in parallel with the samples. The insulin, leptin, and adiponectin concentrations in the samples were calculated in reference to the corresponding standard curves and expressed as nanograms per milliliter.

2.4. Measurement of liver markers of oxidative stress

Liver MDA, GSH, and GSSG (glutathione disulfide) concentrations and SOD activity were measured in duplicate using commercial kits purchased from Cayman Chemical (Ann Arbor, MI). Liver tissue was homogenized in 250 μL radioimmunoprecipitation buffer containing protease inhibitor and centrifuged at 1600*g* for 10 minutes at 4°C. The

Table 1
Comparison of average food intake among treatment groups

Group	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6
NC	20.09 ± 0.71[a]	18.76 ± 0.52[a]	20.59 ± 0.53[a]	19.09 ± 0.62[a]	21.80 ± 1.27[a]	20.62 ± 0.76[a]
HFC	14.69 ± 0.54[b]	14.04 ± 0.51[b]	14.61 ± 0.54[b]	14.26 ± 0.50[bc]	15.27 ± 0.56[b]	15.86 ± 0.86[b]
HFDC	17.88 ± 0.56[c]	16.65 ± 0.57[ab]	17.06 ± 0.71[bc]	16.81 ± 0.52[b]	18.15 ± 0.58[c]	17.18 ± 0.59[b]
BBR50	17.42 ± 0.98[c]	17.06 ± 0.76[a]	17.09 ± 0.86[c]	15.40 ± 0.90[bc]	16.66 ± 0.73[bc]	14.99 ± 0.91[b]
BBR100	15.26 ± 0.55[b]	16.80 ± 1.23[a]	15.53 ± 0.74[b]	14.23 ± 0.58[c]	15.39 ± 0.72[b]	15.05 ± 0.66[b]
BBR150	15.71 ± 0.68[bc]	15.88 ± 0.80[ab]	17.14 ± 0.76[c]	15.87 ± 0.75[b]	16.51 ± 0.74[bc]	16.64 ± 0.70[b]

Data are presented as means ± SEM (in grams per day; n = 12 for each group). For each week, values bearing different superscript letters are different ($P < .05$).

supernatant was collected and measured for SOD activity and MDA concentration according to the kit instructions. The SOD activity and MDA concentrations were calculated in reference to the corresponding standard curves and expressed as units per gram and micromoles per gram of liver tissue, respectively.

For the liver GSH and GSSG analyses, homogenized tissue was centrifuged at 10 000*g* for 15 minutes at 4°C, and the supernatant was removed and stored on ice. Protein was removed using metaphosphoric acid (5 g metaphosphoric acid in 50 mL dH_2O) and 4 mol/L triethanolamine (531 μL triethanolamine in 469 μL dH_2O) reagents. The deproteinated supernatant was then used to measure the GSH concentrations by following the kit instructions. For the liver GSSG analysis, liver GSH was first derivatized with 1 mol/L 2-vinylpyridine (108 μL 2-vinylpyridine in 892 μL ethanol). The assay was then performed as per GSH. In reference to the standard curves, the concentrations of GSH and GSSG were calculated and expressed as micromoles per gram of liver tissue.

2.5. Measurement of plasma inflammatory cytokines

Plasma TNF-α was quantified in duplicate using a commercial enzyme-linked immunosorbent assay kit purchased from Pierce Endogen (Rockford, IL). To the anti–TNF-α antibody-coated 96-well plate, 50 μL of samples or standards was added, followed by a 1-hour incubation at room temperature. After 3 washes with the wash buffer, 50 μL of a biotinylated antibody reagent was introduced, and the plate was incubated for 1 hour at room temperature. A second wash cycle was performed, followed by addition of 100 μL of substrate 3,3′,5,5′-tetramethylbenzidine and a 30-minute incubation in a dark room. The reaction was then terminated by adding 100 μL of stop solution, and absorbances were read at 450 nm. Plasma TNF-α concentrations were calculated in reference to the standard curve and expressed as picograms per milliliter.

2.6. Statistical analysis

All rats were able to complete the study, and data analyses were performed by 1-way analysis of variance (ANOVA) using SAS 9.1 (SAS Institute, Cary, NC). Except for body weight and food intake, all values were logarithmically transformed. Differences between treatment means were determined by pairwise comparisons using the least squares means test, with $P < .05$ indicating statistical significance. Results are presented as means ± SEM.

3. Results

3.1. Berberine lowered food intake with no effect on body weight

Rats fed the high-fat diet had reduced food intake compared with rats fed the normal diet (Table 1). Streptozotocin-induced diabetic rats had increased food intake at weeks 1 and 5 compared with the high-fat control rats. After BBR treatment with the 100-mg/kg/d dose, food intake was lowered ($P < .05$) and significantly different from the diabetic control at weeks 1, 4, and 5. Berberine treatment with the 50- and 150-mg/kg/d doses did not alter food intake significantly in diabetic rats.

The high-fat diet increased body weight compared with the normal control diet throughout the treatment period (Table 2). Diabetic rats had lower ($P < .05$) body weights than the high-fat control and normal control rats during the entire study. Berberine treatment did not significantly

Table 2
Comparison of average body weight among treatment groups

Group	Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6
NC	304.7 ± 5.8[ac]	322.8 ± 6.0[a]	336.8 ± 6.2[a]	354.6 ± 5.9[a]	366.2 ± 6.8[a]	365.2 ± 6.1[a]	379.0 ± 6.8[a]
HFC	326.2 ± 7.5[a]	347.8 ± 8.6[b]	365.9 ± 8.8[b]	384.6 ± 9.9[b]	404.0 ± 10.8[b]	409.2 ± 12.2[b]	424.7 ± 13.3[b]
HFDC	284.0 ± 8.9[b]	302.1 ± 8.8[c]	309.3 ± 10.3[c]	317.2 ± 12.3[c]	322.8 ± 14.4[c]	330.5 ± 15.6[c]	338.8 ± 17.3[c]
BBR50	269.8 ± 9.9[bc]	300.5 ± 5.9[c]	307.3 ± 6.9[c]	317.3 ± 8.6[c]	322.8 ± 10.6[c]	326.5 ± 11.4[c]	329.4 ± 14.8[c]
BBR100	288.3 ± 7.8[b]	302.1 ± 8.3[c]	312.9 ± 9.9[c]	326.0 ± 9.5[c]	334.5 ± 11.7[c]	335.6 ± 13.0[ac]	342.3 ± 14.2[ac]
BBR150	292.5 ± 8.0[b]	301.2 ± 5.4[c]	306.8 ± 8.4[c]	316.8 ± 9.0[c]	324.1 ± 11.3[c]	324.5 ± 11.7[c]	336.6 ± 16.3[c]

Data are presented as means ± SEM (in grams; n = 12 for each group). For each week, values bearing different superscript letters are different ($P < .05$).

improve body weights during the first 5 weeks of treatment. At week 6, animals administered BBR at 100 mg/kg/d had increased body weights that became similar to the normal control rats, yet remained lower ($P < .05$) than the high-fat control rats.

3.2. Berberine showed hypoglycemic effects in diabetic rats

The effect of BBR on glucose control was assessed by semifasting blood glucose values, which were measured weekly after a 5-hour fasting period. As shown in Fig. 1, BBR treatment at both the 50- and 100-mg/kg/d doses was able to lower ($P < .05$) semifasting blood glucose levels at week 6. This trend, although deemed to be significant at marginal levels, was also noted consistently at all other time points ($P = .05$, .09, .05, .06, and .07 at weeks 1, 2, 3, 4, and 5, respectively) in response to the 100-mg/kg/d dose. The 100-mg/kg/d dose tended to show a consistently stronger hypoglycemic effect than the 50-mg/kg/d dose, but the differences between these 2 doses did not reach statistical significance. The 150-mg/kg/d dose did not, however, show a significant effect on semifasting blood glucose levels.

In line with the semifasting blood glucose results, rats treated with the 100-mg/kg/d dose showed significantly decreased ($P < .05$) overnight-fasting blood glucose levels compared with the diabetic control (Fig. 2). The 50- and 150-mg/kg/d doses did not, however, show a significant improvement in blood glucose levels.

3.3. Berberine showed insulin-sensitizing effects in diabetic rats

To determine the insulin-sensitizing capability of BBR, OGTT was performed during the last week of treatment. Compared with the diabetic control, the 100-mg/kg/d dose group showed a significant improvement in oral glucose tolerance (Fig. 3). Rats in this group had lower ($P < .05$) blood glucose levels at time points 0, 15, and 120 minutes and marginally decreased blood glucose levels 30 minutes post–oral glucose loading ($P = .06$). The 150-mg/kg/d dose group had lower ($P < .05$) blood glucose levels than the diabetic controls at time points 0, 15, and 30 minutes and also lower blood glucose levels 60 minutes post–oral glucose loading at a marginal significance level ($P = .06$). The 50-mg/kg/d dose did not show any significant effects on oral glucose tolerance.

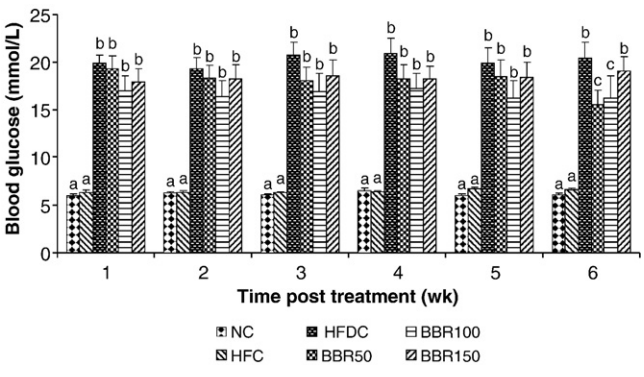

Fig. 1. Effect of BBR treatment on semifasting (5 hours) blood glucose levels in rats. Data were analyzed by 1-way ANOVA with least squares mean post hoc testing to indicate differences among the groups. Values were presented as means ± SEM (n = 12 for each group). Means with different superscript letters are different ($P < .05$).

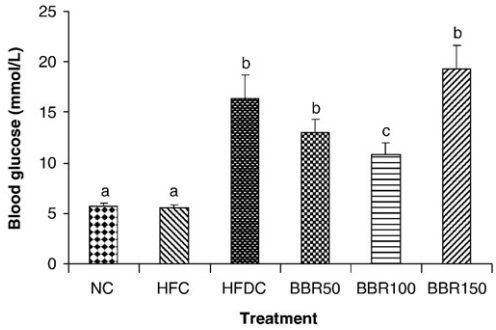

Fig. 2. Effect of BBR on fasting (12 hours) blood glucose levels in diabetic rats. Data were analyzed by 1-way ANOVA with least squares mean post hoc testing to indicate differences among the groups. Values were presented as means ± SEM (n = 12 for each group). Means with different superscript letters are different ($P < .05$).

3.4. Effect of BBR on plasma levels of inflammatory biomarkers

The analysis of plasma levels of the inflammatory markers revealed that TNF-α was significantly increased in response to the high-fat diet feeding and further elevated with the induction of diabetes (Table 3). After BBR treatment, plasma TNF-α levels decreased to levels that were similar to those in the high-fat control, although the

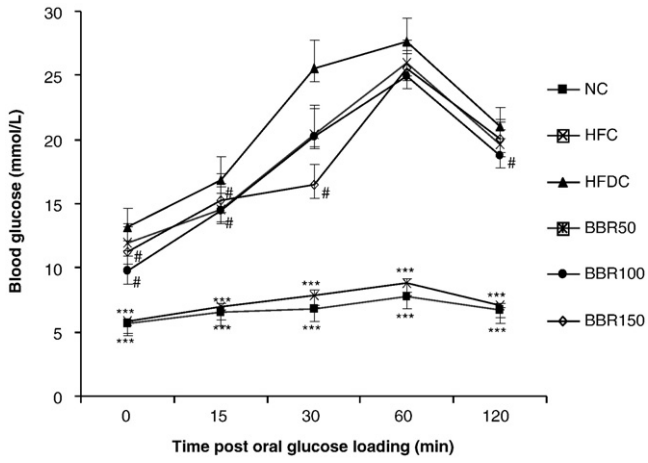

Fig. 3. Effect of BBR on OGTT blood glucose levels in diabetic rats. Data were analyzed by 1-way ANOVA with least squares mean post hoc testing to indicate differences among the groups. Values were presented as means ± SEM (n = 12 for each group). Means with different superscript letters are different. $^{\#}P < .05$, different from the HFDC group; $^{***}P < .0001$, lower than the HFDC and the 3 treatment groups.

Table 3
Comparison of plasma TNF-α levels among the treatment groups

Group	TNF-α
NC	0.285 ± 0.147^{a}
HFC	6.297 ± 1.298^{b}
HFDC	11.824 ± 1.628^{c}
BBR50	11.726 ± 2.404^{bc}
BBR100	9.749 ± 2.347^{bc}
BBR150	8.065 ± 1.702^{bc}

Data are presented as means ± SEM (in picograms per milliliter; n = 12 for each group). Values bearing different superscript letters are different ($P < .05$).

effect was not significantly different from the diabetic control ($P = .15$). Similar effects were seen in plasma levels of C-reactive protein (data not shown).

3.5. Effect of BBR on plasma lipids and free fatty acids

The analysis of plasma lipids including total cholesterol and triglycerides showed a significant increase in response to the high-fat diet (data not shown). With diabetes development, total cholesterol was further elevated in all 3 BBR treatment groups, but not to a significantly different level. Triglyceride levels were unchanged in response to diabetes development. Plasma FFA levels were unchanged in response to the high-fat diet, but increased with diabetes development (data not shown). A marginally significant ($P = .08$) decrease in FFA levels was observed in response to the 50-mg/kg/d dose but not to the 100- or 150-mg/kg/d dose doses.

3.6. Berberine had no effect on plasma insulin, leptin, or adiponectin levels in diabetic rats

The high-fat diet increased leptin and decreased adiponectin levels while showing no effect on plasma insulin levels compared with the normal control (Table 4). In comparison to the high-fat control, rats with diabetes showed significantly decreased ($P < .05$) plasma insulin and leptin levels, whereas plasma adiponectin levels were unaffected. Berberine treatment at all 3 doses showed insignificant effects on plasma insulin, leptin, and adiponectin levels.

Table 4
Comparison of plasma insulin, leptin, and adiponectin levels among the treatment groups

Group	Insulin	Leptin	Adiponectin
NC	4.20 ± 1.47^{a}	1.071 ± 0.135^{a}	$11\,639.5 \pm 948.1^{a}$
HFC	5.16 ± 0.86^{a}	2.226 ± 0.269^{b}	9166.0 ± 673.5^{b}
HFDC	1.28 ± 0.43^{b}	0.779 ± 0.090^{a}	7862.3 ± 687.0^{b}
BBR50	1.39 ± 0.34^{b}	1.110 ± 0.139^{a}	8107.3 ± 517.5^{b}
BBR100	1.32 ± 0.29^{b}	0.881 ± 0.085^{a}	8135.7 ± 634.1^{b}
BBR150	1.51 ± 0.28^{b}	0.965 ± 0.182^{a}	9228.2 ± 626.1^{b}

Data are presented as means ± SEM (in nanograms per milliliter; n = 12 for each group). For each parameter, values bearing different superscript letters are different ($P < .05$).

3.7. Berberine did not affect oxidative stress biomarkers in diabetic rats

Oxidative stress status was assessed by measuring the liver concentrations of MDA, GSH, and GSSG and SOD activity. The high-fat diet decreased both GSH and GSSG concentrations with no significant effect on the ratio of GSH to GSSG, MDA levels, or SOD activity (Table 5). Neither diabetes nor BBR treatment significantly affected the concentrations or activity of these oxidative stress biomarkers.

4. Discussion

In the present study, the effects of BBR on diabetes were assessed using a high-fat diet– and STZ-induced diabetic rat model. The effect of BBR on insulin secretion, insulin sensitivity, and blood glucose levels were investigated, as well as food intake and body weight, the adipokines leptin and adiponectin, and parameters relating to oxidative stress and inflammation. The results showed significant hypoglycemic BBR effects at week 6, along with marginally significant hypoglycemic effects at all other time points, as evidenced by semifasting blood glucose levels. The hypoglycemic effects of BBR were further supported by the overnight-fasting blood glucose levels measured after 6 weeks of treatment. The observed hypoglycemic effects of BBR are supported by the results of previous in vitro [26,27] and in vivo [21,28] studies. Berberine treatment also improved insulin sensitivity as evidenced by the OGTT results, in line with a previous study [16]. However, the magnitudes of fasting blood glucose and oral glucose tolerance improvements were smaller than previously reported [5,16]. In agreement with improved blood glucose and oral glucose tolerance, food intake in diabetic rats was gradually decreased after BBR treatment, became similar with the normal high-fat control after 3 weeks, and was sustained thereafter. As diabetes generally causes increases of food intake, reductions of food intake after BBR treatments are indicative of improvement of diabetic conditions [29].

The plasma insulin levels observed in the present study revealed no significant BBR effects on insulin secretion in the high-fat diet– and STZ-induced diabetic rats during the 6-week treatment period. It is well known that injection of a high dose of STZ (>45 mg/kg) significantly damages the ability of pancreatic β-cells to synthesize and secrete insulin in rats [30]. Consequently, these animals develop impaired insulin response to food ingestion and glucose loading, and accordingly, impaired glucose uptake/utilization capabilities [16,31], mimicking human type 1 diabetes mellitus. Recently, multiadministration of low doses of STZ was used to induce moderate impairment of insulin secretion, more characteristic of later-stage T2DM [15]. In the present study, a single low dose of STZ (35 mg/kg) was used to induce diabetes in rats that were also fed a high-fat diet for

Table 5
Comparison of liver concentrations of MDA, GSH, GSSG, GSH:GSSG, and SOD activity among the treatment groups

Group	MDA	GSH	GSSG	GSH:GSSG	SOD
NC	0.132 ± 0.008[a]	2.23 ± 0.20[a]	0.20 ± 0.025[a]	13.78 ± 2.43[a]	27 124.7 ± 3322.5[ab]
HFC	0.128 ± 0.014[a]	1.63 ± 0.21[b]	0.14 ± 0.018[b]	11.50 ± 1.37[a]	17 117.3 ± 1716.2[b]
HFDC	0.119 ± 0.009[a]	1.47 ± 0.14[b]	0.15 ± 0.017[ab]	11.28 ± 1.49[a]	22 905.3 ± 3377.1[ab]
BBR50	0.108 ± 0.007[a]	1.46 ± 0.15[b]	0.14 ± 0.013[ab]	11.24 ± 1.31[a]	17 148.9 ± 1705.9[b]
BBR100	0.127 ± 0.014[a]	1.43 ± 0.21[b]	0.16 ± 0.019[ab]	11.37 ± 2.72[a]	20 491.2 ± 2593.6[ab]
BBR150	0.129 ± 0.017[a]	1.62 ± 0.17[b]	0.15 ± 0.018[ab]	11.26 ± 1.17[a]	24 025.9 ± 2202.5[a]

Data are presented as means ± SEM (in micromoles per gram of liver tissue [MDA, GSH, GSSG, and GSH:GSSG] and units per gram of liver tissue [SOD]; n = 12 for each group). For each parameter, values bearing different superscript letters are different ($P < .05$).

2 weeks before the STZ injection. Although STZ-induced diabetes significantly decreased plasma insulin levels, BBR treatment did not lead to any improvements. Because STZ induces diabetes by damaging β-cells, the results of the present study suggest that BBR is unable to help the pancreas to recover β-cell function or regenerate β-cells in rats experiencing low-dose STZ–induced diabetes under the current experimental conditions. The effect of BBR on insulin secretion is controversial. Despite studies supporting the insulinotropic capabilities of BBR [3,28], our study did not show any insulinotropic effect in accordance with a recent study in rats [32]. By contrast, an in vitro study showed reductions of insulin secretion in pancreatic β-cells after BBR treatment [33]. The reason for these discrepancies is unclear and may be due to the different experimental conditions and research models used, including cell models [33,34], islets models [35], and animal models [15,21,31].

It is well known that adipokines play an important role in energy and glucose metabolism [9,10], and that their circulating levels are associated with obesity and diabetes [13]. Blood leptin levels are increased by obesity and decreased by diabetes [11]. Obesity, insulin resistance, and diabetes are all linked to reduced levels of adiponectin [13]. In line with these studies, the present study demonstrated that high-fat diet feeding induces significant increases in leptin and decreases in adiponectin and that, by contrast, diabetes reduces blood leptin levels in rats, in agreement with a previous study [11]. Interestingly, diabetes did not change plasma adiponectin levels. Moreover, treatment of diabetic rats with BBR did not result in significant alterations in either leptin or adiponectin levels, indicating that leptin nor adiponectin were involved in the improvement of insulin sensitivity by BBR supplementation. A recent study showed that BBR increased adiponectin in 3T3-L1 adipocytes [36], and another indicated that BBR reduced leptin in the same cells [14]. However, there are no data available relating to the effect of BBR on plasma/serum levels of these 2 adipokines in animals.

In general, high-fat feeding or obesity results in elevated oxidative stress, which is further increased with the development of diabetes [1]. In accordance with these studies, the high-fat feeding in the present study caused reductions of SOD and GSH, indicating that oxidative stress was increased by high-fat consumption. However, the single low-dose STZ–induced diabetes did not significantly affect the levels of these oxidative stress biomarkers including MDA, GSSG, or the GSH to GSSG ratio. The insignificant BBR effects on these oxidative stress biomarkers may also be due to the use of a different animal model in the present study compared with others [1,37], in which oxidative stress was increased in diabetic rats and reduced after BBR treatment. On the other hand, results of our study suggest that the improved blood glucose and oral glucose tolerance by BBR may be independent from oxidative stress.

The insignificant effect of BBR on the aforementioned parameters may suggest that BBR improves glucose control through other mechanisms, such as anti-inflammation and reduction of FFA, leading to improved insulin sensitivity. It is reported that in insulin-resistant states of obesity and T2DM, the plasma concentration of TNF-α is increased [38-40]. In the present study, the high-fat feeding resulted in a significant elevation in plasma levels of the inflammatory biomarkers TNF-α and C-reactive protein, and diabetes resulted in further elevation. Berberine treatment at the doses of 100 and 150 mg/kg/d tended to reduce plasma levels of TNF-α. Several studies have demonstrated that elevated plasma FFA levels are related to the development of insulin resistance and T2DM [18,19]. We observed in STZ-induced diabetic rats that BBR supplementation appeared to decrease plasma FFA levels. As BBR did not show significant effects on insulin secretion or oxidative stress, the improved ability of BBR to control blood glucose might be related to its anti-inflammatory and FFA-lowering effects. Although not tested in the current study, the effect of BBR on blood glucose levels and oral glucose tolerance might also be attributed to other mechanisms related to insulin action/sensitivity, such as dipeptidyl peptidase–IV inhibition [41], enhanced action of glucagon-like peptide–1 [26], and increased glucose metabolism through induction of glycolysis [42]. It is also possible that BBR improved insulin sensitivity by up-regulating insulin receptor expression [43-45].

In conclusion, the present study has demonstrated the antidiabetic hypoglycemic and insulin-sensitizing effects of BBR in a rat model of STZ-induced diabetes, however, the magnitudes were smaller than those reported by others. Berberine supplementation reversed the increase of food intake in diabetic rats, with no effect on body weight. The mechanism of action of BBR on diabetes may be attributed

in part to its anti-inflammatory and FFA-lowering effects, as well as other mechanisms, which await further investigation.

Acknowledgment

This study was supported by funding from the National Research Council of Canada. We would like to extend our appreciation to Drs Jonathan Spears and Bart Gragtmans for their technical support and training of the research staff involved in the study. We would also like to thank the staff at the Atlantic Veterinary College animal care facility at the University of Prince Edward Island for their assistance in animal care.

References

[1] Zhou J, Zhou S, Tang J, Zhang K, Guang L, Huang Y, et al. Protective effect of berberine on beta cells in streptozotocin- and high-carbohydrate/high-fat diet–induced diabetic rats. Eur J Pharmacol 2009;606:262-8.

[2] Yi P, Lu FE, Xu LJ, Chen G, Dong H, Wang KF. Berberine reverses free-fatty-acid–induced insulin resistance in 3T3-L1 adipocytes through targeting IKKbeta. World J Gastroenterol 2008;14:876-83.

[3] Cheng Z, Pang T, Gu M, Gao AH, Xie CM, Li JY, et al. Berberine-stimulated glucose uptake in L6 myotubes involves both AMPK and p38 MAPK. Biochim Biophys Acta 2006;1760:1682-9.

[4] Hsieh YS, Kuo WH, Lin TW, Chang HR, Lin TH, Chen PN, et al. Protective effects of berberine against low-density lipoprotein (LDL) oxidation and oxidized LDL-induced cytotoxicity on endothelial cells. J Agric Food Chem 2007;55:10437-45.

[5] Tang LQ, Wei W, Chen LM, Liu S. Effects of berberine on diabetes induced by alloxan and a high-fat/high-cholesterol diet in rats. J Ethnopharmacol 2006;108:109-15.

[6] Hwang JM, Wang CJ, Chou FP, Tseng TH, Hsieh YS, Lin WL, et al. Inhibitory effect of berberine on *tert*-butyl hydroperoxide–induced oxidative damage in rat liver. Arch Toxicol 2002;76:664-70.

[7] Kim KS, Rhee HI, Park EK, Jung K, Jeon HJ, Kim JH, et al. Anti-inflammatory effects of Radix Gentianae Macrophyllae (Qinjiao), Rhizoma Coptidis (Huanglian) and Citri Unshiu Pericarpium (Wenzhou migan) in animal models. Chin Med 2008;3:10.

[8] Jeong HW, Hsu KC, Lee JW, Ham M, Huh JY, Shin HJ, et al. Berberine suppresses proinflammatory responses through AMPK activation in macrophages. Am J Physiol Endocrinol Metab 2009; 296:E955-64.

[9] Cuellar MJ, Giner RM, Recio MC, Manez S, Rios JL. Topical anti-inflammatory activity of some Asian medicinal plants used in dermatological disorders. Fitoterapia 2001;72:221-9.

[10] Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89:2548-56.

[11] Schmidt MI, Duncan BB, Vigo A, Pankow JS, Couper D, Ballantyne CM, et al. Leptin and incident type 2 diabetes: risk or protection? Diabetologia 2006;49:2086-96.

[12] Diez JJ, Iglesias P. The role of the novel adipocyte-derived hormone adiponectin in human disease. Eur J Endocrinol 2003;148:293-300.

[13] Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 2006;17:4-12.

[14] Choi BH, Ahn IS, Kim YH, Park JW, Lee SY, Hyun CK, et al. Berberine reduces the expression of adipogenic enzymes and inflammatory molecules of 3T3-L1 adipocyte. Exp Mol Med 2006; 38:599-605.

[15] Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet–fed and low-dose streptozotocin–treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res 2005;52:313-20.

[16] Zhang M, Lv XY, Li J, Xu ZG, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp Diabetes Res 2008;704045:2008.

[17] Tripathy D, Mohanty P, Dhindsa S, Syed T, Ghanim H, Aljada A, et al. Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. Diabetes 2003;52:2882-7.

[18] Huffman KM, Shah SH, Stevens RD, Bain JR, Muehlbauer M, Slentz CA, et al. Relationships between circulating metabolic intermediates and insulin action in overweight to obese, inactive men and women. Diabetes Care 2009;32:1678-83.

[19] Kashyap SR, Ioachimescu AG, Gornik HL, Gopan T, Davidson MB, Makdissi A, et al. Lipid-induced insulin resistance is associated with increased monocyte expression of scavenger receptor CD36 and internalization of oxidized LDL. Obesity (Silver Spring) 2009.

[20] Belfort R, Mandarino L, Kashyap S, Wirfel K, Pratipanawatr T, Berria R, et al. Dose-response effect of elevated plasma free fatty acid on insulin signaling. Diabetes 2005;54:1640-8.

[21] Leng SH, Lu FE, Xu LJ. Therapeutic effects of berberine in impaired glucose tolerance rats and its influence on insulin secretion. Acta Pharmacol Sin 2004;25:496-502.

[22] Ward WK, Beard JC, Halter JB, Pfeifer MA, Porte Jr D. Pathophysiology of insulin secretion in non–insulin-dependent diabetes mellitus. Diabetes Care 1984;7:491-502.

[23] Zhou L, Yang Y, Wang X, Liu S, Shang W, Yuan G, et al. Berberine stimulates glucose transport through a mechanism distinct from insulin. Metabolism 2007;56:405-12.

[24] Chen QM, Xie MZ. Studies on the hypoglycemic effect of *Coptis chinensis* and berberine. Yao Xue Xue Bao 1986;21:401-6.

[25] Gao CR, Zhang JQ, Huang QL. Experimental study on berberine raised insulin sensitivity in insulin resistance rat models. Zhongguo Zhong Xi Yi Jie He Za Zhi 1997;17:162-4.

[26] Lu SS, Yu YL, Zhu HJ, Liu XD, Liu L, Liu YW, et al. Berberine promotes glucagon-like peptide–1 (7-36) amide secretion in streptozotocin-induced diabetic rats. J Endocrinol 2009;200:159-65.

[27] Kim SH, Shin EJ, Kim ED, Bayaraa T, Frost SC, Hyun CK. Berberine activates GLUT1-mediated glucose uptake in 3T3-L1 adipocytes. Biol Pharm Bull 2007;30:2120-5.

[28] Ko BS, Choi SB, Park SK, Jang JS, Kim YE, Park S. Insulin sensitizing and insulinotropic action of berberine from *Cortidis rhizoma*. Biol Pharm Bull 2005;28:1431-7.

[29] Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. Lancet 2002;359:824-30.

[30] Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001;50:537-46.

[31] Ar'Rajab A, Ahren B. Long-term diabetogenic effect of streptozotocin in rats. Pancreas 1993;8:50-7.

[32] Wang C, Li J, Lv X, Zhang M, Song Y, Chen L, et al. Ameliorative effect of berberine on endothelial dysfunction in diabetic rats induced by high-fat diet and streptozotocin. Eur J Pharmacol 2009;620:131-7.

[33] Zhou L, Wang X, Shao L, Yang Y, Shang W, Yuan G, et al. Berberine acutely inhibits insulin secretion from beta-cells through 3′,5′-cyclic adenosine 5′-monophosphate signaling pathway. Endocrinology 2008; 149:4510-8.

[34] Wang ZS, Lu FE, Chen G, Xu LJ, Wang KF, Zou X. Effect of berberine on insulin secretion and glucokinase activity of NIT-1 cells. Yao Xue Xue Bao 2007;42:1045-9.

[35] Wang ZQ, Lu FE, Leng SH, Fang XS, Chen G, Wang ZS, et al. Facilitating effects of berberine on rat pancreatic islets through modulating hepatic nuclear factor 4 alpha expression and glucokinase activity. World J Gastroenterol 2008;14:6004-11.

[36] Gu W, Zeng WH, Hu HY. Effects of berberine on adiponectin mRNA expression in 3T3-L1 adipocyte. Zhongguo Zhong Yao Za Zhi 2005; 30:286-8.

[37] Singh J, Kakkar P. Antihyperglycemic and antioxidant effect of *Berberis aristata* root extract and its role in regulating carbohydrate metabolism in diabetic rats. J Ethnopharmacol 2009;123:22-6.

[38] Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003;112: 1821-30.

[39] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87-91.

[40] Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol 2004;25:4-7.

[41] Lankas GR, Leiting B, Roy RS, Eiermann GJ, Beconi MG, Biftu T, et al. Dipeptidyl peptidase IV inhibition for the treatment of type 2 diabetes: potential importance of selectivity over dipeptidyl peptidases 8 and 9. Diabetes 2005;54:2988-94.

[42] Yin J, Gao Z, Liu D, Liu Z, Ye J. Berberine improves glucose metabolism through induction of glycolysis. Am J Physiol Endocrinol Metab 2008;294:E148-E156.

[43] Kong WJ, Zhang H, Song DQ, Xue R, Zhao W, Wei J, et al. Berberine reduces insulin resistance through protein kinase C-dependent up-regulation of insulin receptor expression. Metabolism 2009;58:109-19.

[44] Wang YX, Wang YP, Zhang H, Kong WJ, Li YH, Liu F, et al. Synthesis and biological evaluation of berberine analogues as novel up-regulators for both low-density-lipoprotein receptor and insulin receptor. Bioorg Med Chem Lett 2009;19:6004-8.

[45] Zhang H, Wei J, Xue R, Wu JD, Zhao W, Wang ZZ, et al. Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression. Metabolism 2009;59:285-92.