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A PRELIMINARY INVESTIGATION OF THE MECHANISMS UNDERLYING THE EFFECT OF BERBERINE IN PREVENTING HIGH-FAT DIET-INDUCED INSULIN RESISTANCE IN RATS

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Berberine exerts insulin resistance-improving effects, the underlying mechanism of which is not well understood. We herein aimed to examine the effects of berberine on mediators of insulin signaling in pancreatic β- and α- islet cells and hepatocytes using a rat obesity model. Rats were fed the following diets for 22 weeks: normal control (NC); normal+berberine (NC+BBR 200 mg/kg/day); high-fat (HF); HF+BBR_1 (BBR 100 mg/kg/day); HF+BBR_2 (BBR 200 mg/kg/day). Metabolic parameters were assessed and mediators of insulin signaling were quantified by immunohistochemistry. The HF diet significantly increased body weight (BW), visceral fat (VF), the visceral fat to BW ratio (VF/BW), and insulin resistance index in the HF group compared with the NC group. Both doses of BBR significantly reduced HF diet-induced increases in BW, VF, and VF/BW. IR and IRS-1 expression in β-cells was significantly lower in the HF group, but not the HF+BBR groups, compared with the NC and NC+BBR groups. Glucagon expression in α-cells was significantly higher in the HF group compared with all other groups. IR expression in α-cells was significantly lower in the HF group compared with the NC, NC+BBR, and HF+BBR_2 groups. IR expression in hepatocytes was significantly lower in the HF group compared with all groups. Our preliminary findings suggest that berberine may ameliorate the development of insulin resistance by differentially preventing alterations in expression of IR, IRS-1, and glucagon in β-cells, α-cells, and hepatocytes.

Key words: *berberine, diabetes, hepatocytes, high-fat diet, insulin resistance, pancreatic α-cells, pancreatic β-cells*

INTRODUCTION

Obesity can lead to insulin resistance, impaired glycemic control, and ultimately type 2 diabetes (1). Insulin and glucagon are the key mediators of glycemic control. Insulin secretion is primarily regulated by glucose, which enters pancreatic β-cells *via* glucose transporter 1 where it is metabolized to adenosine triphosphate (ATP) (1). The subsequent increase in the ATP/adenosine diphosphate ratio leads to the closure of ATP-sensitive K^+ channels, membrane depolarization, opening of Ca^{2+} channels, influx of Ca^{2+}, and consequent secretion of insulin. Glucagon secretion from pancreatic α-cells is mediated by a number of factors, including glucose concentrations, somatostatin, autonomic innervation, and glucagon-like peptides-1 and -2. These factors act through various intracellular pathways to regulate glucagon secretion.

The causes of insulin resistance are complex; however, excessive levels of circulating free fatty acids (FFAs) appear to play an important role, particularly in the liver, skeletal muscle, and pancreatic islet cells (2). Insulin signal transduction pathways are active in pancreatic islet cells (3-5). High levels of FFAs are thought to be associated with decreased expression of insulin receptor substrate-1 (IRS-1) in peripheral tissues and impaired glucose utilization, resulting in subsequent hyperglycemia and hyperinsulinemia (6-9). Findings from other studies have also indicated that dyslipidemia can decrease the expression of insulin receptor (IR), IRS-1, and IRS-2 in pancreatic islet cells, thus impairing the insulin signal transduction pathway (10-13). Impaired insulin signal transduction not only affects signalling in islet β-cells, but can also lead to increased glucagon secretion because insulin negatively regulates glucagon secretion from α-cells through the IRS -1-PI3K pathway (14). These changes, both insulin resistance and increased glucagon secretion, are thought to be associated with the development of type 2 diabetes mellitus (15, 16).

Current treatments for insulin resistance/type 2 diabetes, such as biguanides and thiazolidinediones, can improve insulin sensitivity; however, their clinical application is limited to a certain extent because of associated side effects, in particular weight gain, hypoglycemia, and gastrointestinal disturbance (17). Hence, alternative treatment options are needed. Berberine, an isoquinoline alkaloid, has been shown to exert glucose-lowering, lipid-regulating, and insulin resistance-improving effects in humans and animals (18, 19). These effects of berberine may be mediated, at least in part, by altered insulin signaling in pancreatic β- and α-cells. Importantly, treatment with berberine does not appear to have any significant side effects (18). The precise mechanism of action of berberine is

unclear; however, multiple potential mechanisms have been described. For instance, berberine has been reported to improve the sensitivity of skeletal muscle and liver cells to insulin by increasing IR expression (20, 21). Further, there is also evidence to suggest that berberine may alter the makeup of gut microbia and consequently help alleviate insulin resistance by decreasing the exogenous antigens and increasing intestinal short-chain fatty acid concentrations (22). We have also recently reported that berberine can reduce palmitate-associated lipotoxicity in pancreatic β-cells *in vitro* (23). This finding is relevant in the context of type 2 diabetes and insulin resistance because lipotoxicity (mediated by increased FFA levels) is an important cause of insulin resistance. Further research is needed to determine the extent to which berberine affects mediators of insulin signaling in pancreatic islet β- and α-cells and thereby better understand the mechanism of action of berberine.

The aim of this preliminary study was to examine the effects of berberine on mediators of insulin signaling in pancreatic islet β- and α-cells and hepatocytes isolated from rats fed a high-fat diet known to induce obesity and insulin resistance. We hypothesized that berberine would ameliorate the development of insulin resistance and that this may be reflected by differential expression of: insulin, IR, and IRS-1 in pancreatic β-cells; glucagon, IR, IRS-1, and IRS-2 in pancreatic α-cells; and IR, and IRS-1 in hepatocytes. We also examined key metabolic and biochemical indicators of insulin, glucose, and FFA homeostasis.

MATERIALS AND METHODS

Animals

A total of 49 eight-week-old male Wistar rats (SPF grade; weight: 160 to 180 g) were purchased from Chengdu ASUS Animal Ltd. (Sichuan, China) and housed (temperature: 18 to 25°C; 12 hour light/dark cycle) for 2 weeks at the SPF animal facility of West China Hospital of Sichuan University. This study was approved by the Animal Ethics Committee of Sichuan University.

Establishment of obesity model

The 49 rats were randomly assigned to one of the following five groups: (1) normal control (NC: n=10); (2) normal diet +BBR (NC+BBR: 200 mg/kg/day, n=9); (3) high-fat diet (HF: n=10); (4) high-fat diet +BBR_1 (HF+BBR_1: 100 mg/kg/day, n=9); (5) high-fat diet +BBR_2 (HF+BBR_2: 200 mg/kg/day, n=11). Rats in the NC group were fed regular chow and were gavaged daily with 2.4 mL/kg distilled water. Rats in the NC+BBR group were fed regular chow (per rats in the NC group) and were gavaged daily with 200 mg/kg BBR. Rats in the HF group were fed a high-fat diet and were gavaged daily with 2.4 mL/kg distilled water. Rats in the HF+BBR_1 were fed a high-fat diet (per rats in the HF group) and were gavaged daily with 100 mg/kg BBR. Rats in the HF+BBR_2 were fed a high-fat diet (per rats in the HF group) and were gavaged daily with 200 mg/kg BBR. Rats were fed their respective diets for 22 weeks. Body weight (BW) was measured once every three days and the dose of berberine was adjusted according to BW.

The dose of berberine was chosen with reference to previous studies, which have reported optimal glucose lowering effects with 100 mg/kg and 200 mg/kg of BBR (24, 25).

Berberine hydrochloride (99.5%) was purchased from Hongyi Bio-Engineering Co., Ltd (Sichuan, China). Regular chow contained 20% protein, 5% fat, 54.5% carbohydrate, 7% crude ash (mainly containing minerals, oxides, or salts), 4.5% crude fiber, and 9% water (Chengdu Huashuo Animal, Chengdu, China). There were 3.43 kcal/g in the regular chow diet (total energy: carbohydrates =68%, fats =18%, and protein =14%). The high-fat diet was a modification of a previously described diet (26). The diet contained 10% egg yolk powder (comprising 30% protein, 60% fat, and 10% carbohydrate (Bozhou Hongri Egg Products, Anhui, China)), 20% pure lard (Zhumadian Dingsheng Food Co., Ltd, Henan, China), 10% sugar, and 60% regular chow. There were 4.96 kcal/g in the high-fat feed (total energy: carbohydrates =44%, fats =48%, and proteins =8%).

Oral glucose tolerance testing and sample preparation

After 21weeks, 5 rats were randomly selected from each group and fasted for 8 hours. Fasting blood glucose (FBG) and 2-h postprandial blood glucose (2hPG: after gavage with a 20% glucose solution ([2 g/kg]) concentrations were measured in tail vein blood samples using a blood glucose meter (Abbott Laboratories, Chicago, IL).

After 22 weeks, all rats were fasted for 8 hours and anesthetized by intraperitoneal injection of 10% chloral hydrate (2 mL/kg). Thoracotomy was performed and blood was sampled from the heart. An aliquot of blood was used for immediate assessment of FBG concentrations, while serum was separated from the remaining blood and stored at –60°C for later measurement of fasting insulin (FINS), fasting glucagon (FGLC), triglyceride (TG), and FFA concentrations.

The pancreas and liver were then immediately dissected, fixed in 4% formaldehyde, and embedded in paraffin for immunohistochemical detection of pancreatic insulin, IR, and IRS-1, and hepatic IR and IRS-1.

Visceral fat (VF), including the epididymal fat pad, retroperitoneal perirenal fat, and mesenteric fat, was dissected and dried on filter paper. The weight was recorded, and the ratio of VF to BW (VF/BW) was calculated. The insulin resistance index (HOMA-IR: FINS×FBG/22.5) was calculated to evaluate the degree of peripheral insulin resistance.

Assays

1. Serum of fasting insulin, fasting glucagon, free fatty acids, and triglycerides

Serum FINS and FGLC concentrations were measured using commercial enzyme-linked immunosorbent kits (Shanghai Xitang Biotechnology, Shanghai, China) following the manufacturer's instructions. Serum FFA concentrations were measured by colorimetry using a commercially available kit (Mike Technology, Sichuan, China) following the manufacturer's instructions. Serum TG concentrations were measured using the GPO-PAP method with a commercially available kit (Mike Technology) following the manufacturer's instructions.

2. Immunohistochemistry

Five samples of pancreatic tissue and liver tissue were taken from each group for immunohistochemical staining. After fixation, dehydration, and clearing, samples were embedded in paraffin. Consecutive slices of the same sample were used for the detection of: insulin, IR, and IRS-1 in pancreatic β-cells; glucagon, IR, IRS-1, and IRS-2 in pancreatic α-cells; and IR and IRS-1 in hepatocytes.

The tissue slices were incubated with rabbit anti-mouse insulin polyclonal antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-mouse IR polyclonal antibody (1:200; Beijing Biosynthesis Biotechnology, Beijing, China), rabbit anti-mouse IRS-1 polyclonal antibody (1:100; Signalway Antibody, Pearland, TX), rabbit glucagon monoclonal antibody (1:2000;

Abcam Plc., Cambridge, UK), or rabbit IRS-2 polyclonal antibody (1:100; Beijing Biosynthesis Biotechnology) at 37°C for 45 min. A universal rabbit/mouse secondary antibody (Dako, Carpinteria, CA) was used. A DAB substrate solution was then added. Pancreatic α-cells were identified by immunohistochemical double staining whereby samples were incubated with an alkaline phosphatase labeled glucagon-specific antibody and subsequently, a horseradish peroxidase-labeled insulin receptor-specific antibody. Glucagon and insulin receptor-positive cells (*i.e.*, α-cells) in the peripheral islet were stained dark black in color.

The slides were observed under a phase contrast microscope (Olympus IX70, Olympus Corporation, Tokyo, Japan). For pancreatic samples, 3 to 7 intact islets were selected for immunohistochemical analysis. Immunohistochemical images were analyzed using Image-Pro Plus 5.0 image analysis software (MediaCybernetics, Inc., Bethesda, MD). Protein expression was quantified by assessing the integrated optical density (IOD), which is the average OD × the total area. For each tissue slice, the IOD for the particular protein was assessed in 5 different visual fields. The mean IOD for rats in the same group was taken as the expression level for the protein of interest.

Additional liver tissue slices were stained with hematoxylin and eosin (H&E), using standard procedures, to examine cellular pathology.

Statistical analysis

Data are presented as means ± standard deviations. Normally distributed data were compared between groups using one-way analysis of variance with Bonferroni adjustment. HOMA-IR data were non-normally distributed and were compared among groups using Kruskal-Wallis test and Mann-Whitney U test for post-hoc, pair-wise comparisons. Statistical significance was indicated by $P<0.05$. The statistical significance level was adjusted to $P<0.01$ (0.05/5) for post-hoc, pair-wise comparisons. All statistical analyses were performed using SPSS 18.0 statistical software (SPSS Inc, Chicago, IL).

RESULTS

Body weight

Table 1 summarizes the changes in BW for each group of rats during the study period. At Week 22, BW was significantly higher in the HF group compared with the NC group ($P<0.01$), whereas there was no significant difference in BW between the NC and NC+BBR groups. At Week 22, BW was significantly lower in the HF+BBR_1 and HF+BBR_2 groups compared with the HF group ($P<0.01$).

Metabolic and biochemical parameters

Table 2 summarizes the metabolic and biochemical parameters at Week 22 for each group of rats. All parameters except for FBG were significantly different among the 5 groups. VF, VF/BW, and HOMA-IR were significantly higher in the HF group compared with the NC group ($P<0.01$). Compared with the NC group, BBR alone did not significantly alter any metabolic or biochemical parameters, except for VF/BW ($P<0.01$). VF and VF/BW were significantly lower in both HF+BBR groups compared with the HF group ($P<0.01$). FGLC was significantly higher in the HF group compared with the NC+BBR and HF+BBR_1 groups ($P<0.01$).

Immunohistochemistry

1. Pancreatic islet beta cells

There were no significant between group differences in insulin expression (*Table 3*). In contrast, both IR and IRS-1 expression was significantly lower in the HF group compared with the NC and NC+BBR groups ($P<0.01$). IR and IRS-1 expression was numerically higher in both HF+BBR groups compared with the HF group, although the between group differences were not significant. Representative microscopic images demonstrating (brown) staining for insulin, IR, and IRS-1 are shown in *Fig. 1* (note: staining in the center of the islet indicates β-cells).

2. Pancreatic islet alpha cells

Glucagon expression was significantly higher in the HF group compared with all other groups ($P<0.01$, *Table 3*). IR expression was significantly lower in the HF group compared with the NC, NC+BBR, and HF+BBR_2 groups ($P<0.01$). There were no significant between group differences in IRS-1 and IRS-2 expression. Representative microscopic images demonstrating (brown) staining for glucagon, IR, IRS-1, and IRS-2 are shown in *Fig. 2* (note: staining along the peripheral of the islet indicates α-cells).

Table 1. Comparison of changes in body weight during the 22 week study for rats fed different diets.

Week	NC (n=10)	NC+BBR (n=9)	HF (n=10)	HF+BBR_1 (n=9)	HF+BBR_2 (n=11)	*P*
Week 0 (g) 0 (g)	306.30 ± 38.44	308.00 ± 17.13	312.56 ± 16.40	298.89 ± 10.78	296.27 ± 25.67	0.596
Week 4 (g) 4 (g)	375.1 ± 56.68	405.11 ± 31.34	390.33 ± 29.28	394.89 ± 22.19	391.27 ± 28.23	0.522
Week 8 (g) 8 (g)	456.50 ± 51.97	467.78 ± 37.16	474.22 ± 34.74	442.67 ± 33.20	453.55 ± 29.11	0.461
Week 12 (g) 12 (g)	485.7 ± 53.70	487.11 ± 31.94	518.67 ± 38.41	469.67 ± 31.25	486.64 ± 30.82	0.134
Week 16 (g) 16 (g)	497.3 ± 45.75	486.78 ± 27.82	547.33 ± 29.83[b]	484.89 ± 32.90[c]	491.45 ± 30.75[c]	0.002*
Week 20 (g) 20 (g)	529.10 ± 36.18	524.00 ± 31.55	602.44 ± 37.68[ab]	511.78 ± 21.70[c]	521.91 ± 15.17[c]	<0.001*
Week 22 (g) 22 (g)	569.30 ± 55.34	535.22 ± 32.94	651.89 ± 40.86[ab]	530.44 ± 50.67[c]	541.82 ± 36.04[c]	<0.001*

Data are presented as mean ± standard deviation and were compared among groups by one-way analysis of variance with Bonferroni adjustment.

Abbreviations: NC, normal control diet; NC+BBR, normal diet + berberine (200 mg/kg/day); HF+BBR_1, high-fat diet + berberine (100 mg/kg/day); HF+BBR_2, high-fat diet + berberine (200 mg/kg/day).

* Indicates an overall significant difference, $P<0.05$; [a] Indicates a significant difference compared with the NC group, $P<0.01$ (0.05/5); [b] Indicates a significant difference compared with the NC+BBR group, $P<0.01$ (0.05/5); [c] Indicates a significant difference compared with the HF group, $P<0.01$ (0.05/5).

Table 2. Comparison of metabolic and biochemical parameters for rats fed different diets for 22 weeks.

Parameter	NC (n=10)	NC+BBR (n=9)	HF (n=10)	HF+BBR_1 (n=9)	HF+BBR_2 (n=11)	*P*
VF, g	23.19 ± 8.42	19.86 ± 5.01	62.73 ± 10.64[ab]	36.61 ± 9.33[bc]	40.36 ± 12.66[abc]	<0.001*
VF/BW	0.040 ± 0.012	0.037 ± 0.008[a]	0.096 ± 0.013[ab]	0.069 ± 0.014[abc]	0.074 ± 0.019[abc]	<0.001*
FGLC, mmol/L	382.1 ± 67.63	296.44 ± 55.77	524.45 ± 144.89[b]	323.1 ± 84.94[c]	378.54 ± 135.9	0.001*
FBG, mmol/L	4.6 ± 0.68	4.88 ± 0.54	5.18 ± 0.55	5.03 ± 0.69	4.77 ± 0.62	0.274
2hPG, mmol/L	6.06 ± 0.85	5.66 ± 0.8	8.02 ± 0.84[b]	6.02 ± 1.04	6.72 ± 1.1	0.006*
TG, mmol/L	1.7 ± 0.51	1.36 ± 0.38	1.93 ± 0.59	1.34 ± 0.28	1.68 ± 0.44	0.035*
FFA, umol/L	360.21 ± 74.08	361.29 ± 66.47	556.59 ± 135.32[b]	419.52 ± 82.17	417.6 ± 88.79	<0.001*
FINS, mIU/L	13.58 ± 3.22	13.18 ± 6.41	22.69 ± 4.78	16.06 ± 7.81	16.2 ± 6.28	0.006*
HOMA-IR	2.79 ± 0.77	2.83 ± 1.28	5.29 ± 1.44[ab]	3.51 ± 1.61	3.42 ± 1.29	0.009*

Data are presented as mean ± standard deviation and were compared among groups by one-way analysis of variance with Bonferroni adjustment, except for FGLC and HOMA-IR data, which were compared by Kruskal-Wallis test and Mann-Whitney U test for post-hoc pair-wise comparisons.

Abbreviations: NC, normal control diet; NC+BBR, normal diet + berberine (200 mg/kg/day); HF, high fat diet; HF+BBR_1, high-fat diet + berberine (100 mg/kg/day); HF+BBR_2, high-fat diet + berberine (200 mg/kg/day); VF, visceral fat; BW, body weight; FGLC, fasting glucagon; FBG, fasting blood glucose; 2hPG, plasma glucose at two hours after meals; TG, triglyceride; FFA, free fatty acid; FINS, fasting insulin; HOMA-IR, insulin resistance index.

* Indicates an overall significant difference, $P<0.05$; [a] Indicates a significant difference compared with the NC group, $P<0.01$ (0.05/5); [b] Indicates a significant difference compared with the NC+BBR group, $P<0.01$ (0.05/5); [c] Indicates a significant difference compared with the HF group, $P<0.01$ (0.05/5).

Table 3. Expression of insulin-related proteins in pancreatic β and α cells and in hepatocytes from rats fed different diets for 22 weeks.

	NC rats		HF rats			*P*
	NC (n=5)	NC+BBR (n=5)	HF (n=5)	HF+BBR_1 (n=5)	HF+BBR_2 (n=5)	
Islet β cells						
Insulin	428.76 ± 50.27	428.05 ± 35.2	584.54 ± 123.44	478.68 ± 67.6	479.55 ± 60.05	0.020*
IR	20.65 ± 1.38	20.35 ± 2.53	12.4 ± 2.86[ab]	18.2 ± 2.78	18.35 ± 2.16	<0.001*
IRS-1	26.83 ± 2.61	24.78 ± 1.44	16.61 ± 2.38[ab]	21.05 ± 3.91	20.86 ± 4.43	0.001*
Islet α cells						
Glucagon	84.97 ± 7.26	84.93 ± 7.99	120.30 ± 6.88[ab]	83.93 ± 4.40[c]	82.91 ± 7.86[c]	<0.001*
IR	470.72 ± 55.98	415.94 ± 48.04	242.21 ± 57.53[ab]	381.67 ± 82.02	423.94 ± 83.00[c]	<0.001*
IRS-1	491.39 ± 146.36	499.66 ± 129.44	404.69 ± 143.23	404.54 ± 127.89	439.87 ± 108.57	0.673
IRS-2	408.13 ± 92.66	423.96 ± 109.44	239.72 ± 88.46	363.22 ± 80.27	363.42 ± 88.28	0.040*
Hepatocytes						
IR	97.48 ± 4.31	93.19 ± 8.84	64.01 ± 6.38[ab]	84.61 ± 8.3[c]	83.85 ± 9.66[c]	<0.001*
IRS-1	98.95 ± 12.12	96.8 ± 9.01	87.42 ± 9.29	94.92 ± 9.96	92.11 ± 11.52	0.474

Data are presented as mean ± standard deviation (optical density units) and were compared among group by one-way analysis of variance with Bonferroni test for post-hoc pair-wise comparisons.

Abbreviations: NC, normal control diet; NC+BBR, normal diet + berberine (200 mg/kg/day); HF, high fat diet; HF+BBR_1, high-fat diet + berberine (100 mg/kg/day); HF+BBR_2, high-fat diet + berberine (200 mg/kg/day); IR, insulin receptor; IRS-1, insulin receptor substrate-1.

* Indicates an overall significant difference, $P<0.05$; [a] Indicates a significant difference compared with the NC group, $P<0.01$ (0.05/5); [b] Indicates a significant difference compared with the NC+BBR group, $P<0.01$ (0.05/5); [c] Indicates a significant difference compared with the HF group, $P<0.01$ (0.05/5).

3. Hepatocytes

IR expression was significantly lower in the HF group compared with all other groups ($P<0.01$, *Table 3*). In contrast, there were no significant between group differences in IRS-1 expression. Representative microscopic images demonstrating (brown) staining for IR and IRS-1 are shown in *Fig. 3*.

H&E staining revealed obvious fatty degeneration/ steatosis in liver samples from rats in the HF group (*Fig. 4*). No obvious pathological changes were observed in any of the other groups.

DISCUSSION

In this preliminary study, we examined the effects of berberine on changes in mediators of insulin signaling in pancreatic and liver cells isolated from rats fed a high-fat diet known to induce obesity and insulin resistance. In contrast to rats fed the high-fat diet alone for 22 weeks, we found that rats fed the high-fat diet in combination with berberine (100 mg/kg/day or 200 mg/kg/day) did not exhibit any significant increases in body weight, changes in indicators of insulin resistance status, changes in IR and IRS-1 expression in pancreatic β-cells, changes in glucagon or IR

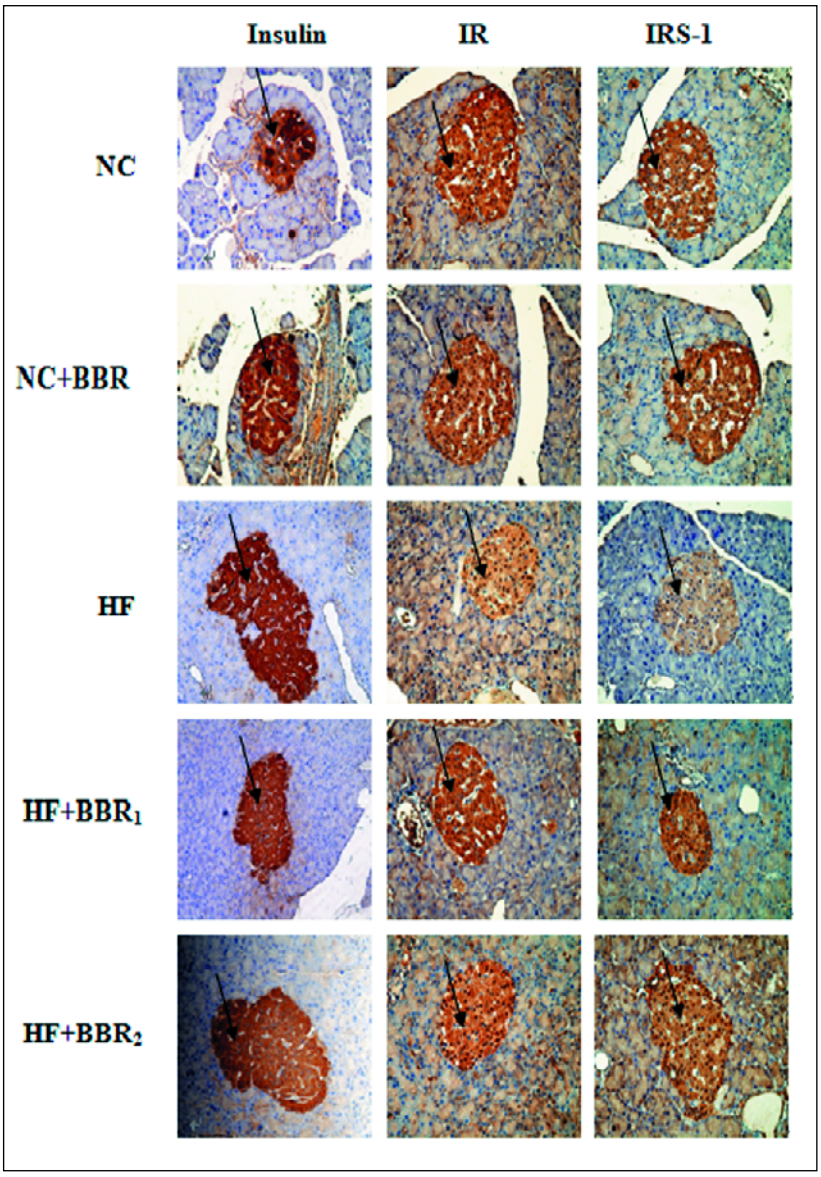

Fig. 1. Immunohistochemical staining of insulin, insulin receptor (IR), and insulin receptor substrate-1 (IRS-1) in islet beta cells from rats fed different diets. NC, normal control diet; NC+BBR, normal diet + berberine (200 mg/kg/day); HF, high fat diet; HF+BBR_1, high-fat diet + berberine (100 mg/kg/day); HF+BBR_2, high-fat diet + berberine (200 mg/kg/day). Arrows indicate positively stained cells. Magnification: ×400.

expression in pancreatic α-cells, or changes in expression of IR in hepatocytes compared with rats fed a normal diet.

Insulin resistance is a common problem in obesity. As such, animal models of obesity-induced insulin resistance have been used to study this condition. Findings from previous studies have shown that obesity-induced insulin resistance can be induced by feeding rats a high-fat diet (27, 28). We found that same to be true in the present study, in which rats fed a high-fat diet for 22 weeks exhibited hallmarks of obesity and insulin resistance, as indicated by significant changes in BW, VF/BW, 2hPG, FFAs, and HOMA-IR.

An important finding of our study was that the majority of the aforementioned biochemical and metabolic changes associated with high-fat diet feeding (indicative obesity and insulin resistance) did not occur when rats fed the high-fat diet were gavaged daily with berberine. Indeed, the only significant change that did occur in these rats was an intermediate increase in VF content and thus the VF/BW ratio. The lack of increase in body weight in rats fed the high-fat diet and gavaged with berberine is a striking finding that could be at least partially explained by berberine increasing vagal nerve stimulation, which in turn is known to suppress food intake (29). Further studies are warranted to examine the effect of berberine on vagal nerve activity. We also observed decreased evidence of hepatic steatosis in rats treated with berberine compared with rats fed the high fat diet. This is an important finding given that hepatic steatosis is a key indicator of nonalcoholic fatty liver disease, which in itself can be challenging to treat (30). Interestingly, the dose of berberine had no obvious impact on the biochemical and metabolic changes (or lack thereof). Whether a higher dose (>200 mg/kg/day) may have prevented the increase in VF content is unclear. It must be noted, however, that both of these doses are higher than the typical dose of berberine given to humans for the treatment of diabetes (500 mg twice daily); hence, conducting further animal studies with higher doses would appear to be of limited relevance. Further studies are, however, needed to determine the optimal dose of berberine for the treatment of diabetes. Our biochemical and metabolic findings are consistent with those from previous studies, in which berberine was found to reduce BW, lower blood glucose and lipid concentrations, and improve insulin resistance (19, 21). The effect of berberine in reducing the extent of the increase in VF content is an important finding, given that VF plays a key role in the development of insulin resistance.

Impaired insulin signalling, of which IR and IRS-1 are key mediators, in pancreatic β-cells is an important cause of insulin

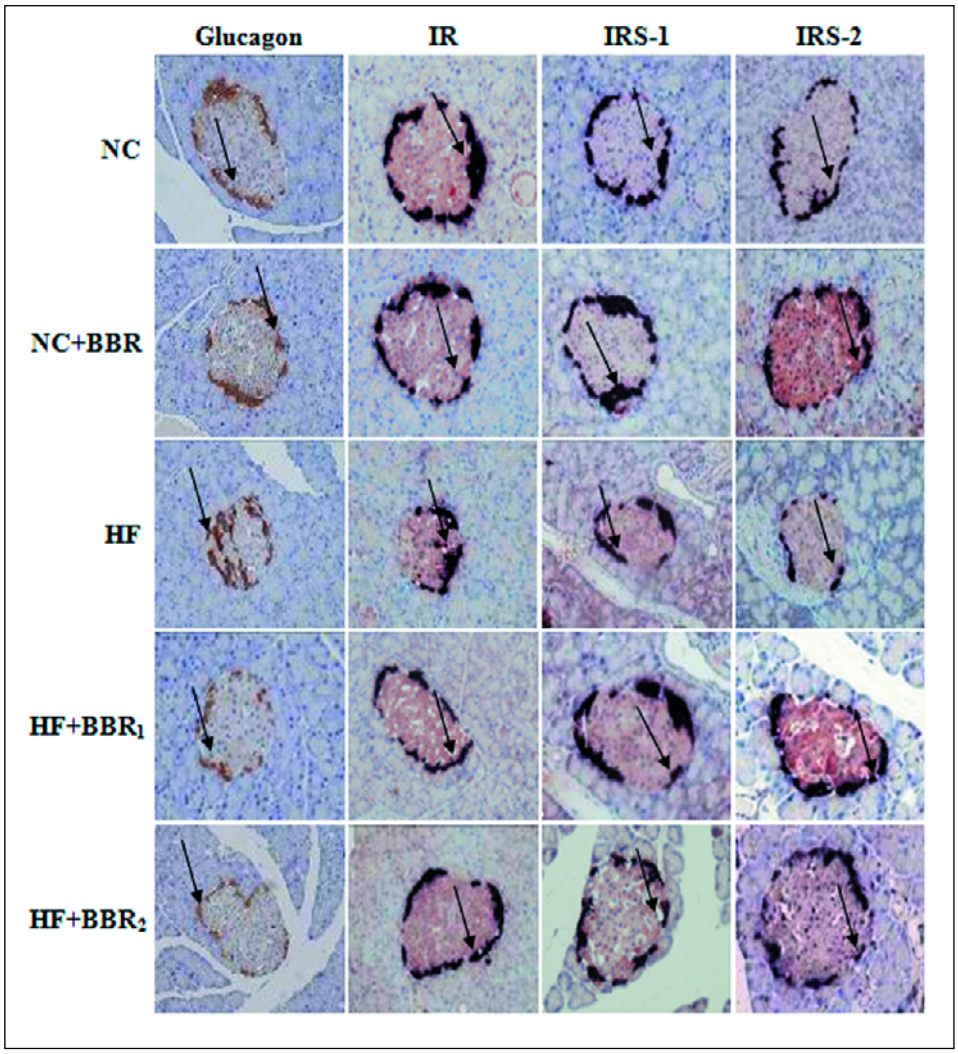

Fig. 2. Immunohistochemical staining of glucagon, insulin receptor (IR), and insulin receptor substrate-1 (IRS-1), and insulin receptor substrate-2 (IRS-2) in islet alpha cells from rats fed different diets. NC, normal control diet; NC+BBR, normal diet + berberine (200 mg/kg/day); HF, high fat diet; HF+BBR_1, high-fat diet + berberine (100 mg/kg/day); HF+BBR_2, high-fat diet + berberine (200 mg/kg/day). Magnification: ×400.

resistance and diabetes (31, 32). In the present study, we found that expression of both IR and IRS-1 were significantly decreased in β-cells from rats fed the high-fat diet compared with rats fed the normal fat diet. These changes are consistent with the previously described biochemical and metabolic changes suggesting that rats fed the high-fat diet were insulin resistant. Notably, these changes in β-cell IR and IRS-1 expression were not detected in rats fed the high-fat diet in combination with berberine. This finding suggests that berberine may have prevented the development of changes consistent with insulin resistance, at least in part, by modulating expression of IR and IRS-1 in β-cells. Given the apparent importance of neurologic modulators (*e.g.*, apelin and vasopressin) in regulating insulin sensitivity (33), it would also be of interest to determine if berberine has any effect on circulating concentrations of these modulators.

To our knowledge, no study to date has examined the effects of berberine on mediators of insulin/glucagon signalling in pancreatic α-cells. In the present study, we found evidence that berberine ameliorates increases in glucagon expression and decreases in IR expression in α-cells with high-fat diet feeding. Our finding regarding the effect of berberine on glucagon expression in α-cells is particularly noteworthy, given that increased glucagon production plays an important role in the development of type 2 diabetes mellitus (15).

In addition to the changes in pancreatic β- and α-cells, we also found that rats fed the high-fat diet had significantly decreased expression of IR in hepatocytes. In contrast, and consistent with lack of change in β-cell IR and IRS-1 expression, hepatocyte IR expression was not increased in rats fed the high-fat diet in combination with berberine. This lack of change in hepatocyte IR expression in rats fed the high-fat diet in combination with berberine may have helped prevent the development of changes consistent with insulin resistance that were observed in rats fed the high-fat diet alone. Interestingly, we did not observe any changes in hepatocyte IRS-1 expression in rats fed the high-fat diet alone. This finding contrasts with that reported by Chen and colleagues (34), who found that IRS-1 protein expression was significantly decreased in rats with dietary-induced obesity and insulin resistance. We suggest that this disparity may be explained by methodological differences *i.e.*, semi-quantitative immunohistochemistry in our study *vs*. Western blotting in Chen and colleagues study (34). Future quantitative real time RT-PCR determination of IRS-1 gene expression may help clarify the disparity in findings.

Our study has several limitations that should be acknowledged. Firstly, being preliminary, our study included a relatively small number of animals per group. This may have limited our ability to detect meaningful between group differences. Secondly, we only used semi-quantitative immunohistochemical methods to assess IR and IRS-1 expression. More comprehensive/quantitative assessment of gene and protein expression is warranted. Thirdly, we cannot comment on how berberine might influence the expression of IR and IRS-1 at the molecular level. Berberine may exert a direct effect on gene

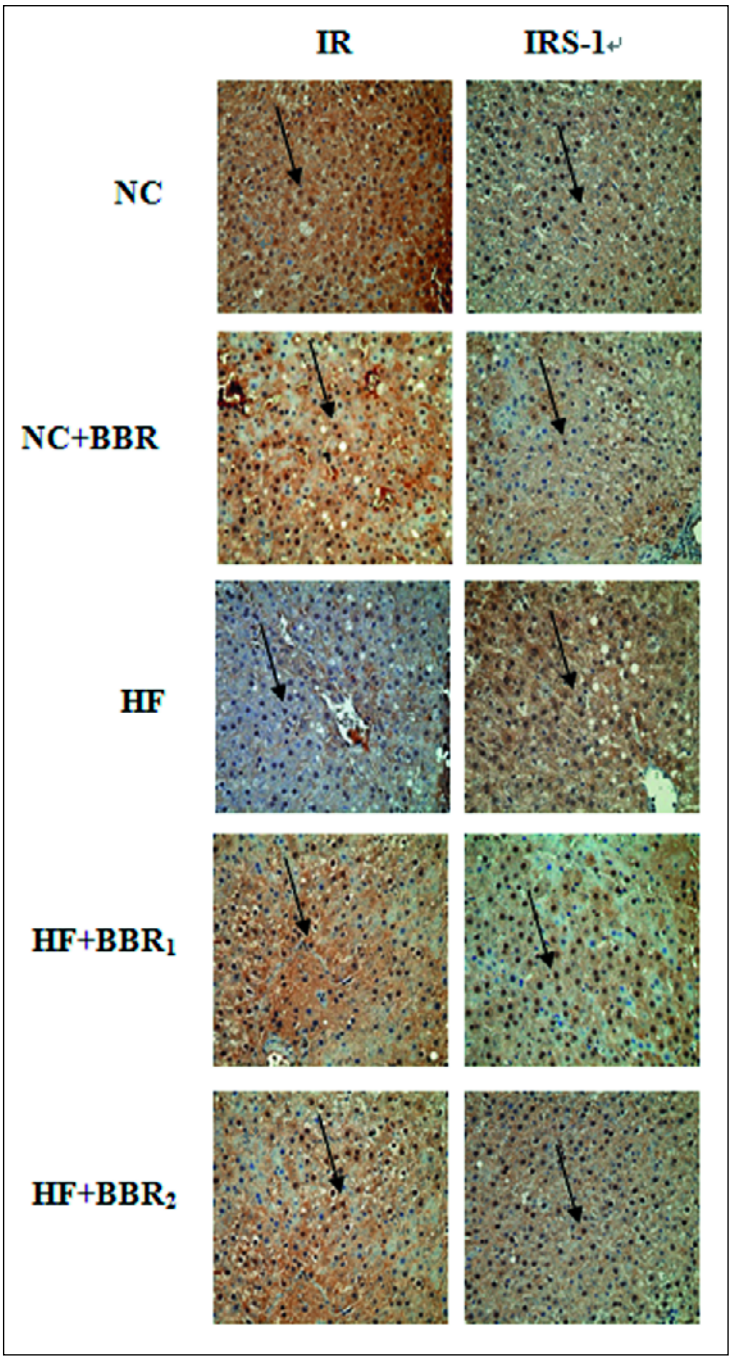

Fig. 3. Immunohistochemical staining of insulin receptor (IR) and insulin receptor substrate-1 (IRS-1) in hepatocytes from rats fed different diets. NC, normal control diet; NC+BBR, normal diet + berberine (200 mg/kg/day); HF, high fat diet; HF+BBR1, high-fat diet + berberine (100 mg/kg/day); HF+BBR2, high-fat diet + berberine (200 mg/kg/day). Arrows indicate positively stained cells. Magnification: ×400.

expression or act in an indirect manner. Further *in vitro* studies are needed to examine these possibilities. Finally, we did not assess expression of phosphorylated IR or IRS-1. As the phosphorylated forms of these proteins are active, we cannot be certain that altered expression of the non-phosphorylated forms of IR and IRS-1 detected in this study would have affected insulin signaling.

In summary, we have found that rats fed a high-fat diet in combination with berberine did not gain significant weight compared with rats fed a normal diet, and did not exhibit biochemical and metabolic changes consistent with insulin resistance. In contrast, rats fed the high-fat diet alone did gain significant weight and exhibited biochemical and metabolic changes consistent with insulin resistance. The effect of berberine in apparently ameliorating the development of insulin resistance in rats fed a high-fat diet may at least in part be related to maintained expression of IR and IRS-1 in pancreatic β-cells, glucagon and IR in pancreatic α-cells, and IR in hepatocytes. Our findings support the continued investigation of berberine as a potential treatment for insulin resistance.

Conflict of interests: None declared.

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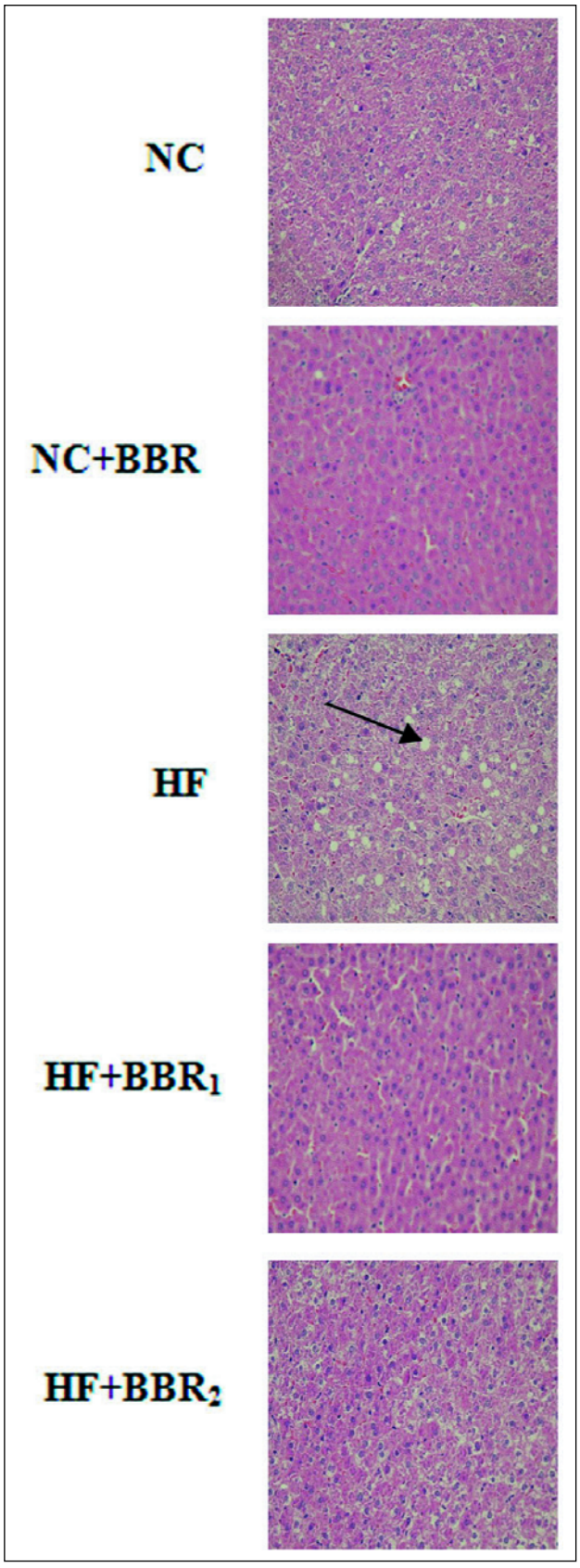

Fig. 4. Hematoxylin and eosin staining of liver samples from rats fed different diets. (A) NC, normal control diet; (B) NC+BBR, normal diet + berberine (200 mg/kg/day;) (C) HF, high fat diet; (D) HF+BBR1, high-fat diet + berberine (100 mg/kg/day); (E) HF+BBR2, high-fat diet + berberine (200 mg/kg/day). Arrow points to a vacuole indicative of steatosis. Magnification: ×400.

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