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Berberine, an Isoquinoline Alkaloid in Herbal Plants, Protects Pancreatic Islets and Serum Lipids in Nonobese Diabetic Mice

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ABSTRACT: Type 1 diabetes (T1D) damages pancreatic islets, gradually causing chronic complications. This study investigated the berberine effect on T1D in vivo. Nonobese diabetic (NOD) mice were grouped and administered 50, 150, and 500 mg of berberine/kg of body weight over 14 weeks using consecutive tube feeding. Changes in pancreatic islets, serum insulin, berberine, and lipid levels were determined. The results showed that berberine supplementation significantly ($P < 0.05$) increased the number of decreased islets and raised serum berberine levels in dose-dependent manners in experimental mice. Berberine supplementation also increased insulin levels, but decreased the ratio of low-density lipoprotein cholesterol (LDL-C)/total cholesterol (TC). Furthermore, serum berberine levels showed a significantly positive correlation with high-density lipoprotein cholesterol (HDL-C) levels and the HDL-C/TC ratio, but a negative correlation with the LDL-C/HDL-C ratio. This study suggests that berberine administration in vivo protects pancreatic islets and serum lipids in NOD mice.

KEYWORDS: berberine, insulin, NOD mice, pancreatic islets, serum lipids

INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disease that progressively induces the selective destruction of pancreatic islet β cells, gradually causing the destruction of at least 80% of insulin-producing β cells.[1] Although the initial cause of pancreatic islet β cells destruction in T1D patients is multiple and remains unclear, chronic inflammation and apoptosis in pancreatic islets play a vital role in the development of T1D. The common chronic damage in diabetes patients is closely related to elevated oxidative/inflammatory activities with a continuum of tissue insults leading to more severe cardiometabolic and interrelated complications,[2] including hyper- and hypoglycemia, ketoacidosis, neuropathy, nephropathy, cardiopathy, and retinopathy.[3] Among diabetic interrelated complications, hypercholesterolemia (especially total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C)) and hyperlipidemia (e.g., triglyceride (TG)) are cardiovascular disease risk factors and associated with enhanced oxidative stress.[4] Atherosclerosis has been linked to the oxidation of lipoproteins, primarily LDL, in the vascular wall.[5] Clearly, hyperlipidemia (high in TC, TG, LDL-C) and in vivo oxidation stress in diabetic patients deteriorate diabetic interrelated complications. However, compounds that show antioxidant and anti-inflammatory potential may prevent pancreatic islet insulin-producing β cells from selective destruction,[1] inhibit islet apoptosis, delay the occurrence of T1D,[6−8] and alleviate diabetic interrelated complications.[2] In traditional Chinese medicine, herbs and their active components have been used in the treatment of diabetes.[9] However, berberine, one of the active components of traditional antidiabetic medicines, exhibits the most promising potential for its potent anti-inflammatory effects.[10] The protective effect of berberine in vivo on T1D remains unclear. This study is the first to investigate the berberine effect in vivo on pancreatic islets, insulin secretion levels, and serum lipids using a nonobese diabetic (NOD) mouse model.

Berberine is an isoquinoline alkaloid from many herbal plants, such as *Hydrastis canadensis* (goldenseal), *Rhizoma coptidis* (Huanglian), *Berberis vulgaris* (barberry), and *Berberis aristata* (tree turmeric), which have been widely used in traditional Chinese medicines for treating diseases via its potent anti-inflammatory activity.[11,12] Berberine is found to have a wide range of pharmacological effects, especially anti-inflammatory effects in vitro and in vivo.[13,14] Berberine increases glucagon-like peptide-1 secretion and biosynthesis in vitro or in vivo,[15] alleviates extracellular matrix accumulation, including intercellular adhesion molecule-1, transforming growth factor-β 1, and fibronectin in the glomerulus of alloxan-induced diabetic mice,[16] and ameliorates endothelial dysfunction of aortas in high-fat diet and STZ-induced diabetic rats.[17] In a word, berberine is a naturally occurring isoquinoline alkaloid[1,10] that may function as an antidiabetic agent through its potent anti-inflammatory and antidiabetic potential. However, its protective effect in vivo on pancreatic islets in T1D patients remains unknown. We investigated the berberine effect on T1D in vivo using NOD mice in this study.

NOD mice are a useful experimental model for T1D disease[18] because the NOD mice spontaneously develop T1D and share several common points with human T1D patients.[19] T1D is a chronic autoimmune disease that is associated with multiple organ inflammation including the pancreas, kidney, liver, and spleen.[20−22] The potent anti-inflammatory activity of berberine in mouse primary splenocytes has been proven.[10] The present study tried to further unravel the berberine administration effect on T1D in vivo.

We hypothesized that berberine has potentially beneficial effects on pancreatic islets in T1D-prone subjects. Thus, the berberine oral supplementation effect for 14 weeks on NOD

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mice was carried out. Changes in pancreatic islets, insulin secretion, serum berberine levels, and lipid profiles including TG, TC, LDL-C, and high-density lipoprotein cholesterol (HDL-C) were determined to unravel the protective effects of berberine on T1D subjects.

MATERIALS AND METHODS

Sample. Berberine ($C_{20}H_{18}NO_4$) is an isoquinoline alkaloid in herbal plants. Berberine chloride (Sigma, Bangalore, India) was purchased at the highest available purity. The purity of berberine was ≥95%.

Experimental Animals and Dietary Groups. Female NOD mice (NOD/ShiLtJ) and female ICR mice (as a normal species control) at 7 weeks old were purchased from The Jackson Laboratory (Bar Harbor, ME) and the BioLasco Taiwan Co., Ltd. (Taipei, Taiwan), respectively. The NOD mice were imported from the United States under the guidance of the Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan, ROC. The experimental mice were maintained at the Department of Food Science and Biotechnology at National Chung Hsing University, College of Agriculture and Natural Resources in Taichung, Taiwan. The mice were housed in stainless steel cages and kept at a controlled temperature (25 ± 2 °C) and ambient humidity (50−75%). Light was maintained on a 12 h dark−light cycle. All mice were kept on a chow diet (laboratory standard diet, Diet MF 18, Oriental Yeast Co., Ltd., Osaka, Japan) to acclimatize for 2 weeks before feeding the AIN-76 experimental diet and water ad libitum. The AIN-76 experimental feed was prepared according to the recommendation of the American Institute of Nutrition AIN-76 that satisfies the nutritional requirement for mouse growth.[23] After this equilibrium period, NOD/LtJ female mice were randomly divided into four groups, including a control group (CO, AIN76 diet), a berberine low-dose group (BL, 50 mg/kg of body weight (BW)), a berberine medium-dose group (BM, 150 mg/kg of BW), and a berberine high-dose group (BH, 500 mg/kg of BW). Female ICR mice were selected as a normal species control (SC, AIN76 diet). During the study period, grouped mice respectively received 0.3 mL extra of deionized water or berberine powder suspended in deionized water by intragastric gavage using a stainless steel feeding syringe for 14 consecutive weeks. Each group consisted of 12 mice. Four mice in the same group were earmarked and housed in an individual stainless steel cage. The experimental mice were randomized over space in the cage. The mouse body weight was measured twice a week during the study period. Some of the NOD mice died due to diabetes during the late experimental period, and thus the data collected from these dead mice were excluded. After the 14 week feeding experiment, the serum was collected and assayed for berberine, insulin, and lipid contents. Pancreatic islets from the experimental mice were isolated and assayed. The animal use protocol listed in this study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), National Chung Hsing University, Taiwan.

Serum and Pancreas Collection. The experimental mice were sacrificed after the 14 week feeding experiment. After fasting for 12 h, the animals were weighed, anesthetized with diethyl ether, and immediately bled using retro-orbital venous plexus puncture to collect blood. Immediately after blood collection, the animals were sacrificed using CO_2 inhalation for tissue collection and analysis. The pancreases were aseptically removed from the experimental mice. The blood was collected into a 1.5 mL vial, allowed to stand for 2 h at room temperature, and then centrifuged at 12000*g* for 15 min at 4 °C to separate the serum. The sera were collected and stored at −30 °C for later analysis.

Isolation and Count of Mouse Primary Pancreatic Islets. Immediately after blood collection, the animals were sacrificed with CO_2. The abdominal cavity was cut open aseptically. Vater's ampula was closed using two pieces of bulldog clamp at duodenum, and then 2 mL of 1.2 mg/mL collagenase solution (type XI collagenase, Sigma, St. Louis, MO) was infused into the pancreas through the common bile duct. The pancreas was aseptically removed from the ICR mice, transferred into a 15 mL of centrifugal tube, and cut into aliquots of 0.2 cm pieces. The mixture was incubated for 20 min at 37 °C in a shaker. The resultant cell mixture was thoroughly mixed with a vortex and passed through a 400 μm stainless screen mesh to another 15 mL centrifugal tube. The screen mesh was washed with 10 mL of sterile Hank's balanced salts solution (HBSS) prepared with 50 mL of 10× HBSS buffer (Hyclone, Logan, UT), 2.5 mL of antibiotic−antimycotic solution (100× PSA, Atlanta Biologicals Inc.) containing 10000 U/mL penicillin, 10 mg/mL streptomycin, and 25 μg/mL amphotericin B in 0.85% saline, 20 mL of 3% bovine serum albumin (BSA; Sigma), 2 mL of 7.5% $NaHCO_3$ (Wako, Osaka, Japan), and 425.5 mL of water. The cell suspension was centrifuged at 400*g* for 7 min. The supernatant was discarded, and the cell pellet was washed with 10 mL of HBSS buffer three times. The pellet was added with an aliquot of 3 mL of Histopaque (density = 1.119 g/mL) (Sigma) to dissolve the cell pellet. The cell suspension was steadily added in turn with an aliquot of 3 mL of Histopaque (density = 1.077 g/mL) (Sigma) and an aliquot of 3 mL of RPMI 1640, avoiding the disturbance of the interface in the tube. The cell suspension was centrifuged at 1610*g* for 20 min. After centrifugation, the pancreatic islets redistributed in the fraction of density 1.077 g/mL. An aliquot of 2.5 mL of top solution was discarded. An aliquot of 3.5 mL of islets (density = 1.077 g/mL) was collected to a 15 mL centrifugal tube, and the collected islets were added to an aliquot of 6.5 mL of HBSS buffer. The islet cell suspension was centrifuged at 400*g* for 7 min. The supernatant was discarded, and the cell pellet washed with 10 mL of HBSS buffer twice. The islet cell pellet was dissolved in 0.5 mL of FBS medium. An aliquot of 20 μLof islet cell suspension was stained with an aliquot of 20 μL of dithizone (DTZ) dye prepared with 0.1 g of DTZ (Fluka, St. Louis, MO), 10 mL of DMSO, and 40 mL of FBS. Pancreatic islets were counted with a hemocytometer.

Berberine Assay Levels in the Serum Using Gradient High-Performance Chromatography (HPLC). To analyze the berberine level in sera of experimental mice, an aliquot of 100 μL of serum sample was first extracted with 500 μL of methanol (Wako). After thorough mixing, the mixture was centrifuged at 12000*g* for 15 min. The supernatant was collected, and the remainder was extracted with 500 μL of methanol again. The berberine extracts were pooled in a 1.5 mL centrifugal tube and evaporated to dryness in an 80 °C water bath. The residue in the tube was dissolved in an aliquot of 100 μL of methanol. The solution was centrifuged at 12000*g* for 15 min. The supernatant was collected and filtered through a 0.22 μm filter (Minisar SRP4, PTFE membrane, Sartorius, Goettingen, Germany). The filtrate sample was stored at −30 °C until use. The berberine chloride (Sigma, Bangalore, India) was dissolved in methanol to prepare a 100 μM berberine standard. The berberine standard was filtered through a 0.22 μm filter (PTFE membrane) and stored at −30 °C until use.

The berberine levels in mouse sera were determined using the HPLC method. For HPLC analysis, the sample solution was ultrasonically degassed before use. Mobile phase A (double-distilled water/tetrahydrofuran (ECHO, Miaoli, Taiwan)/trifluoroacetic acid (Sigma, St. Louis, MO) = 98:2:0.1 (v/v/v)) and mobile phase B (acetonitrile, HPLC grade, ECHO, Miaoli, Taiwan) were filtered through a 0.45 μm filter (Durapore, Millipore, Bedford, MA) under vacuum and ultrasonically degassed before use. The mobile phase flow rate was 1 mL/min. Aliquots of 10 μL of sample solution were subjected to the HPLC analysis. The pump (L-2131, Hitachi, Tokyo, Japan), UV−visible detector (L-2400, Hitachi), and chromatographic separation column (250 × 4.6 mm, 5 μm; Mightsil RP-18 GP250, Kanto Chemical Co., Inc., Tokyo, Japan) were used. The pump was controlled under the program (D-2000 Elite, Hitachi) of a gradient elution starting at mobile phase A 83% and mobile phase B 17% for 7 min, then mobile phase A 75% and mobile phase B 25% for 8 min, mobile phase A 65% and mobile

Table 1. Effects of Berberine Administered at Different Doses on Pancreatic Islet Cell Numbers and Serum Berberine Levels in Experimental Mice through a 14 Consecutive Week Tube Feeding[a]

	group				
item	CO (n = 7)	BL[b]	BM (n = 4)	BH (n = 6)	SC (n = 11)
islet cell number ($\times 10^4$ cells/mice)	5.14 ± 1.95B	7.75 ± 3.77AB	9.00 ± 5.72AB	10.10 ± 4.45A	10.4 ± 3.07*
berberine concentration (μM)	0.00 ± 0.00C	1.15 ± 0.73BC	1.93 ± 1.17AB	3.07 ± 2.06A	0.00 ± 0.00

[a] Values are the mean ± SD. Values among CO, BH, BM, and BL groups within the same row not sharing a common letter are significantly different ($P < 0.05$) from each other as assayed by one-way ANOVA, followed by Duncan's multiple-range test. An asterisk (*) indicates significant difference ($P < 0.05$) between SC and CO groups analyzed by one-way ANOVA, followed by unpaired Student's t test. CO, control; BL, low-dose berberine (50 mg/kg bw); BM, medium-dose berberine (150 mg/kg bw); BH, high-dose berberine (500 mg/kg bw); SC, species control (ICR mice). [b] n = 7 for islet cell number; n = 8 for berberine concentration.

phase B 35% for 5 min, mobile phase A 50% and mobile phase B 50% for 7 min, mobile phase A 0% and mobile phase B 100% for 8 min, and ending at mobile phase A 83% and mobile phase B 17% for 5 min. The detection was at 370 nm. The berberine retention time (RT) was found at 17.7 min.

Mouse Serum Insulin Level Measurement Using Enzyme-Linked Immunosorbent Assay (ELISA). Serum insulin levels were determined using a sandwich ELISA. One hundred microliters of 1:1000 diluted (with phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4, 0.22 μm filtered)) anti-human polyclonal captured antibody (LifeSpan Biosciences, Inc., Seattle, WA) was added to 96-microwell plate wells and incubated overnight at 4 °C. After incubation, the plates were washed three times with ELISA washing buffer solution (PBST, 0.05% Tween 20 in PBS, pH 7.4). To block nonspecific binding, 150 μL aliquots of blocking buffer (1% bovine serum albumin (BSA, USB, Cleveland, OH) in PBS) were added to each well. The plates were incubated at room temperature for 2 h. After incubation, the plates were washed three times with ELISA washing buffer solution. One hundred microliter volumes of sera (1:10 diluted in reagent diluent (PBST)) or mouse insulin standards in duplicate (0.188, 0.5, 1.25, 3.75, and 6.9 ng/mL) (Mercodia AB, Uppsala, Sweden) were added to the 96-microwell plate wells. The plates were incubated overnight at 4 °C. After incubation, the plates were washed four times with ELISA washing buffer solution. One hundred microliters of conjugated horseradish peroxidase (HRP) detection antibody (Abcam, Cambridge, MA) including anti-insulin and anti-proinsulin antibody (1:1000 diluted with reagent diluent) was then added to each well. Plates were incubated at room temperature for 2 h. After incubation, the plates were washed six times with ELISA washing buffer solution. One hundred microliters of substrate solution (tetramethylbenzidine (TMB), Clinical Science Products Inc., Mansfield, MA) were pipeted into the 96-microwell plate wells. Plates were incubated at 37 °C for 1 h to develop color. Fifty microliters of stop solution (2 N H_2SO_4) was added to each well to stop the reaction. The plates were measured for absorbance at 450 nm on a plate reader (ELISA reader, ASYS Hitech GmbH, Salzburg, Austria). The mouse serum insulin levels were determined using the standard curve. The sensitivity of the insulin ELISA kit was <0.188 ng/mL.

Assay of TG, TC, HDL-C, and LDL-C Using Colorimetric Method. The lipid levels in experimental mouse serum were determined using TG, TC, and HDL-C assay kits (Randox, Northern Ireland, U.K.), as well as an LDL-C assay kit (Fortress, Northern Ireland, U.K.), respectively. Serum samples were appropriately diluted. The lipid levels were assayed according to the manufacturer's instructions.

Statistical Analysis. Values are expressed as the mean ± SD. Data were analyzed statistically using one-way ANOVA, if justified by the statistical probability ($P < 0.05$), by Duncan's multiple-range tests or paired Student's t test. The relationship between the serum berberine levels and pancreatic islets number or serum berberine levels and lipid profiles was described using Pearson product-moment correlation coefficient (r). Differences were considered to be statistically significant if $P < 0.05$.

RESULTS AND DISCUSSION

Berberine Supplementation Effects on Pancreatic Islet Cell Numbers and Serum Berberine Levels in Experimental Mice. To evaluate the long-term effects of berberine supplementation on T1D, changes in pancreatic islet cell numbers and serum berberine levels in the experimental mice fed berberine for 14 weeks were determined. ICR mice (SC group) were selected as a normal species control to NOD/LtJ mice. The results showed that the ICR mice had markedly ($P < 0.05$) higher islet cell numbers than NOD mice throughout the experimental period; however, berberine supplementation increased the decreased islet cell numbers in a dose-dependent manner (Table 1). Furthermore, high doses of berberine supplementation significantly ($P < 0.05$) increased the islet cell number compared to the CO group, suggesting that berberine administration protected islet cells from spontaneous injury in NOD mice (Table1).

The serum berberine levels in the experimental mice were determined using HPLC. Representative HPLC chromatograms of sera and berberine standard are shown in Figure 1. The distinguishable peak in the HPLC chromatograms is represented as the retention time (RT). Berberine reflecting RT 17.7 min was easily identified in the HPLC column. Serum berberine levels in individual mice were calculated and compared to the reflected peak berberine standard area. The results showed that serum berberine levels in berberine-administered groups significantly ($P < 0.05$) increased in a dose-dependent manner (Table 1), suggesting that berberine is absorbable via the digestive tract. Quantitative data were accumulated in this study to show how oral berberine supplementation enriched the body and serum concentrations compared to the nonsupplemented group. The bioavailability of berberine supplementation was proven; however, its relative distribution in different body organs remains to be further clarified.

Berberine Supplementation Effects on Serum Insulin Levels in Experimental Mice. Changes in serum insulin levels in the experimental mice were determined to examine the berberine supplementation effect on serum insulin levels. The results showed that ICR mice had slightly ($P > 0.05$) higher insulin levels than NOD mice throughout the experimental period. Berberine administration increased the decreased insulin levels in NOD mice (Figure 2). Obviously, berberine administration in vivo increased insulin secretion levels using pancreatic islets. Unfortunately, serum insulin levels were just slightly, but not significantly ($P > 0.05$)at low and medium doses, changed through the 14 week experimental period. We hypothesized that berberine administration at the indicated concentrations in this study could not fully reverse the spontaneous injury to pancreatic islet β cells in the NOD mice.

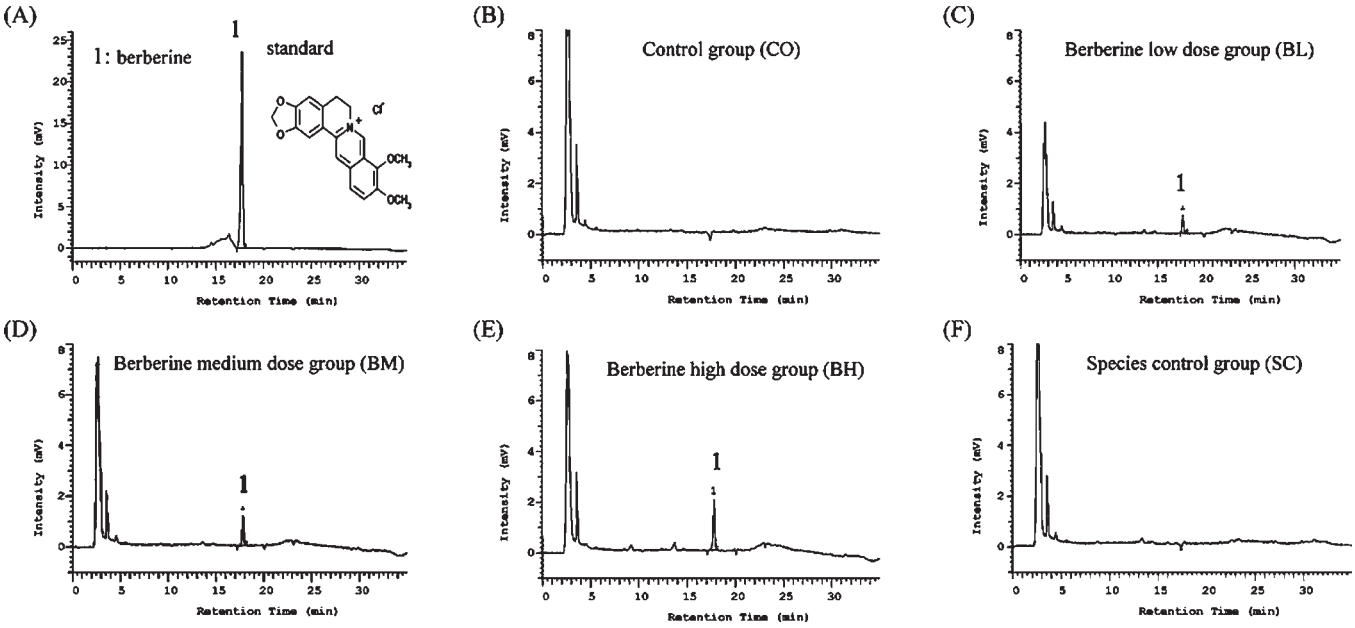

Figure 1. HPLC chromatograms of serum berberine levels in the experimental mice through 14 consecutive weeks of tube feeding. The representative chromatograms are from berberine standard (100 μM) (A), control (B), berberine low dose (50 mg/kg of bw) (C), berberine medium dose (150 mg/kg of bw) (D), berberine high dose (500 mg/kg of bw) (E), and species control (ICR mice) groups (F).

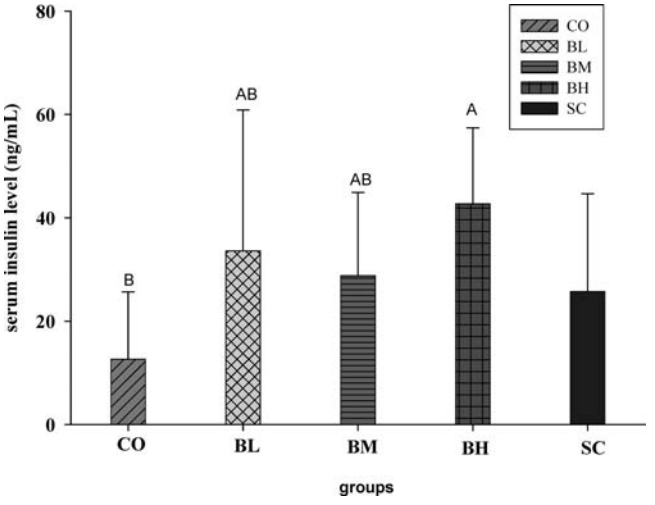

Figure 2. Effects of berberine administered at different doses on fasting serum insulin levels in the experimental mice through 14 consecutive weeks of tube feeding. Values are the mean ± SD. Bars among CO, BH, BM, and BL groups not sharing a common letter are significantly different ($P < 0.05$) from each other as assayed by one-way ANOVA, followed by Duncan's multiple-range test. Data between SC and CO groups were analyzed using one-way ANOVA, followed by unpaired Student's t test. There were no significant differences between the SC and CO groups. SC ($n = 11$), species control (ICR mice); CO ($n = 7$), control; BL ($n = 8$), low-dose berberine (50 mg/kg of bw); BM ($n = 4$), medium-dose berberine (150 mg/kg of bw); BH ($n = 6$), high-dose berberine (500 mg/kg of bw).

Berberine Supplementation Effects on Serum Lipid Levels in the Experimental Mice. The serum lipid levels in the experimental mice are shown in Table 2. Berberine administration did not significantly influence serum lipid levels including serum TG, TC, HDL-C, and LDL-C levels. However, NOD mice (CO group) had significantly ($P < 0.05$) higher ratios of LDL-C/HDL-C and LDL-C/TC compared to ICR mice (SC group) throughout the experimental period, suggesting that relatively higher LDL-C levels existed in NOD mice. Importantly, berberine administration at different doses for 14 weeks markedly ($P < 0.05$) decreased the raised LDL-C/TC ratio. Clearly, decreases in TC, TG, and LDL-C (bad cholesterol) serum levels with an increase in HDL-C (good cholesterol) in vivo are favorable to good health and avoid diabetic complications. Our results suggested that berberine administration improved serum lipid profiles in NOD mice via relatively decreasing LDL-C serum levels.

Correlation among Serum Berberine Levels, Pancreatic Islet Cell Numbers, and Serum Lipid Levels in Berberine-Administered NOD Mice. To further understand the relationship among serum berberine levels, pancreatic islet cell numbers, and serum lipid levels in berberine-administered NOD mice, the correlations between the serum berberine levels and pancreatic islet cell numbers, as well as between the serum berberine levels and serum lipid levels were determined using Pearson's correlation coefficients (r) (Figure 3). The results showed a significantly ($P < 0.05$) positive correlation between the berberine and HDL-C levels (Figure 3C), as well as a significantly positive correlation between the serum berberine levels and HDL-C/TC ratios (Figure 3F) in berberine-administered NOD mice. However, there was a significantly negative correlation between the serum berberine levels and LDL-C/HDL-C ratios (Figure 3E). Furthermore, there was a slightly ($P = 0.083$) positive correlation between the serum berberine levels and pancreatic islet cell numbers in the berberine-administered NOD mice (Figure 3A). Our results further suggested that berberine administration at the indicated concentrations through the 14 week experimental period could not fully protect pancreatic islets from spontaneous injury in the experimental NOD mice.

We directly detected insulin levels rather than c-peptide levels in the experimental mice. Serum fasting glucose levels and oral glucose tolerance test (OGTT) were determined during the

Table 2. Effects of Berberine Administered at Different Doses on Fasting Serum Lipid Levels in the Experimental Mice through 14 Consecutive Weeks of Tube Feeding[a]

	group				
	CO (n = 7)	BL (n = 8)	BM (n = 4)	BH (n = 6)	SC (n = 11)
triglyceride (mmol/L)	1.17 ± 0.77	0.99 ± 0.35	1.19 ± 0.21	1.15 ± 0.12	1.24 ± 0.27
cholesterol (mmol/L)	2.73 ± 0.32	2.84 ± 0.59	2.93 ± 0.12	2.96 ± 0.24	2.70 ± 0.43
HDL-C (mmol/L)	2.15 ± 0.52	2.03 ± 0.33	2.06 ± 0.18	2.24 ± 0.44	2.56 ± 0.59
LDL-C (mmol/L)	2.59 ± 0.23	2.50 ± 0.61	2.54 ± 0.16	2.57 ± 0.14	2.34 ± 0.72
LDL-C/HDL-C	1.27 ± 0.30	1.25 ± 0.28	1.24 ± 0.11	1.21 ± 0.28	0.88 ± 0.28*
HDL-C/TC	0.73 ± 0.18	0.74 ± 0.19	0.74 ± 0.02	0.76 ± 0.18	0.97 ± 0.26
LDL-C/TC	0.95 ± 0.04A	0.88 ± 0.07B	0.87 ± 0.05B	0.88 ± 0.06B	0.81 ± 0.12*

[a] Values are the mean ± SD. Values among CO, BH, BM, and BL groups within the same row not sharing a common letter are significantly different ($P < 0.05$) from each other as assayed by one-way ANOVA, followed by Duncan's multiple-range test. An asterisk (*) indicates significant difference ($P < 0.05$) between SC and CO groups as analyzed by one-way ANOVA, followed by unpaired Student's t test. CO, control; BL, low-dose berberine (50 mg/kg of bw); BM, medium-dose berberine (150 mg/kg of bw); BH, high-dose berberine (500 mg/kg of bw); SC, species control (ICR mice). HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

experimental period (data not shown). Evidence revealed that NOD mice developed type 1 diabetes (Table1 and Figure 2). Furthermore, the experimental design in this study consisting of a control group (CO group) versus intervention groups (BL, BM, and BH groups) could indeed reflect the berberine treatment effect after the 14-week supplementation. Our results indicated that berberine supplementation was easily absorbable via the digestive tract and reflected the corresponding concentrations in the sera of the experimental mice in a dose-dependent manner (Table 1 and Figure 1). Berberine is an isoquinoline alkaloid with moderate water-soluble property rich in many herbal plants, particularly 5−8% berberine in *Rhizoma coptidis* (Huanglian).[11] On the basis of the results from this study, we further suggested that the moderate water-soluble property of berberine enhanced its bioavailability in vivo (Table 1). Increased serum berberine levels provided protective effects from spontaneous pancreatic islet cell injury in NOD mice (Table 1 and Figure 3A). Thus, increased berberine levels raised the decreased islet cell numbers in a dose-dependent manner in the experimental NOD mice (Table 1). Undoubtedly, NOD mice spontaneously developed T1D disease[18] and suffered chronic inflammation resulting in apoptosis, especially in the pancreatic islet β cells.[20] Reasonably, the prevention of pancreatic islet insulin-producing β cells from selective destruction[1] such as anti-apoptotic effect on the islets in the early stage may cure or delay T1D. Unfortunately, β-cell mass in the pancreas was not directly determined in this study. Currently it is difficult to directly diagnose β-cell abnormalities in vivo because β cells are small, as well as deeply and sparsely dispersed within the pancreas. Recently, noninvasive β-cell imaging using a β-cell-specific monoclonal antibody for measurement of β-cell mass in vivo could be provided; however, single-cell resolution cannot yet be achieved to enable discrimination between scattered islets, single β cells, or surrounding tissue. β Cells make up ∼80% of the cells in the pancreatic islets, whereas the remaining 20% of the cells in the islets are mainly α, δ, and pancreatic polypeptide cells. It is believed that islet cell numbers correlate closely with β-cell mass in the pancreas. In our previous studies, berberine showed potent anti-inflammation potential[10] and inhibited streptozotocin-induced apoptosis in mouse pancreatic islets through down-regulating *Bax/Bcl-2* gene expression ratio in vitro (data not shown). Undoubtedly, berberine could protect β cells from cell death in the experimental NOD mice. This work and the findings are identical to the previous study we performed.

After absorption via the digestive tract and reflection in sera (Figure 1C−E), berberine can undoubtedly enter pancreatic islet β cells[24] and exert its anti-apoptotic effect, possibly using its potent anti-inflammatory[10,13,14] and antioxidant activities,[25] as well as down-regulating the *Bax/Bcl-2* gene expression ratio (data not shown). More recently, anthocyanins from Chinese bayberry extract[26] and ginseng extract[27] were found to protect pancreatic islet β cells from hydrogen peroxide (H_2O_2)-induced and cytokine-induced apoptosis, respectively. Importantly, the present study showed that berberine, a natural plant product,[28] in vivo protected pancreatic islets from damage in NOD mice. We hypothesized that berberine in vivo inhibited pancreatic islets apoptosis particularly through its potent anti-inflammation potential.[10] We found that appropriate insulin secretion levels were produced by the increased pancreatic islet β cells (Figures 2 and 3A), although insulin levels were still not significantly ($P > 0.05$) changed through the 14 week experimental period.

We found that NOD mice (CO group) showed significantly ($P < 0.05$) higher LDL-C/HDL-C and LDL-C/TC ratios compared to ICR mice (SC group) throughout the experimental period, suggesting that relatively higher LDL-C levels exist in NOD mice (Table 2). Surprisingly, berberine administration at different doses for 14 weeks markedly ($P < 0.05$) decreased the raised LDL-C/TC ratio, suggesting that berberine administrations improved serum lipid profiles in NOD mice. Further analysis also showed a significantly ($P < 0.05$) positive correlation between berberine and HDL-C levels (Figure 3C), as well as a significantly positive correlation between serum berberine levels and HDL-C/TC ratios (Figure 3F), but a significantly negative correlation between serum berberine levels and LDL-C/HDL-C ratios in NOD mice (Figure 3E). Our results indicated that berberine administration in vivo improved serum lipid profiles in NOD mice via relatively increasing serum HDL-C, but decreasing LDL-C levels. The results further suggested that berberine administration may alleviate cardiometabolic complications in diabetes via improving serum lipid profiles.[2] Berberine was found to inhibit fatty acid synthesis via decreasing the acetyl-CoA carboxylase activity in 3T3-L1 adipocytes;[27] however, the regulatory mechanism of berberine on serum lipid levels and profiles remains to be further clarified. Unfortunately, histological analysis of

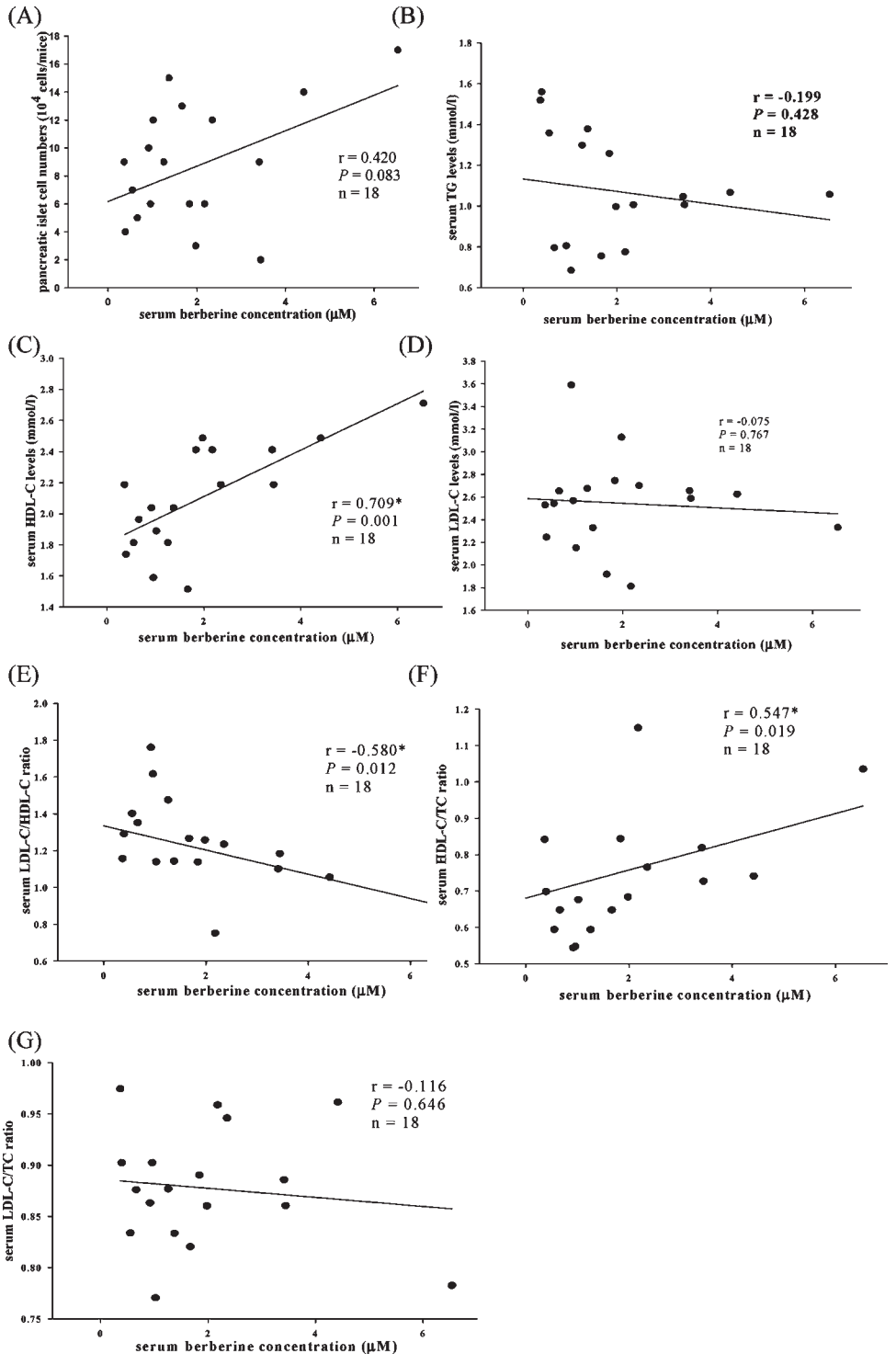

Figure 3. Correlations between serum berberine concentration and pancreatic islet cell numbers (A), serum TG levels (B), serum HDL-C levels (C), serum LDL-C levels (D), serum LDL-C/HDL-C ratio (E), serum HDL-C/TC (F), or serum LDL-C/TC ratio (G) in berberine-administered NOD mice. The correlations are expressed as Pearson product-moment correlation coefficient (r). *, correlation was considered to be statistically significant at $P < 0.05$.

the liver, pancreas, and lung were not performed to further evaluate the protective effects of berberine on T1D-induced organ fibrosis. Berberine administration decreased the ratios of pro-/anti-inflammatory cytokine gene expressions in the liver and kidney of NOD mice, suggesting an anti-inflammatory protection of berberine on visceral organs (data not shown).

Overall, berberine, an isoquinoline alkaloid in herbal plants, was studied for its effect on T1D in vivo for 14 weeks using a NOD mouse model. The results showed that berberine supplementation significantly increased the decreased pancreatic islet cell numbers and enhanced serum berberine levels in dose-dependent manners. Berberine supplementation increased insulin

levels, but decreased the ratio of LDL-C/TC. Serum berberine levels showed a significantly positive correlation with HDL-C levels and the HDL-C/TC ratio, but showed a negative correlation with the LDL-C/HDL-C ratio. This study suggests that berberine administration in vivo protects pancreatic islets and serum lipids in NOD mice.

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ABBREVIATIONS USED

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NOD mice, nonobese diabetic mice; TC, total cholesterol; TG, triglyceride; T1D, type 1 diabetes.

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