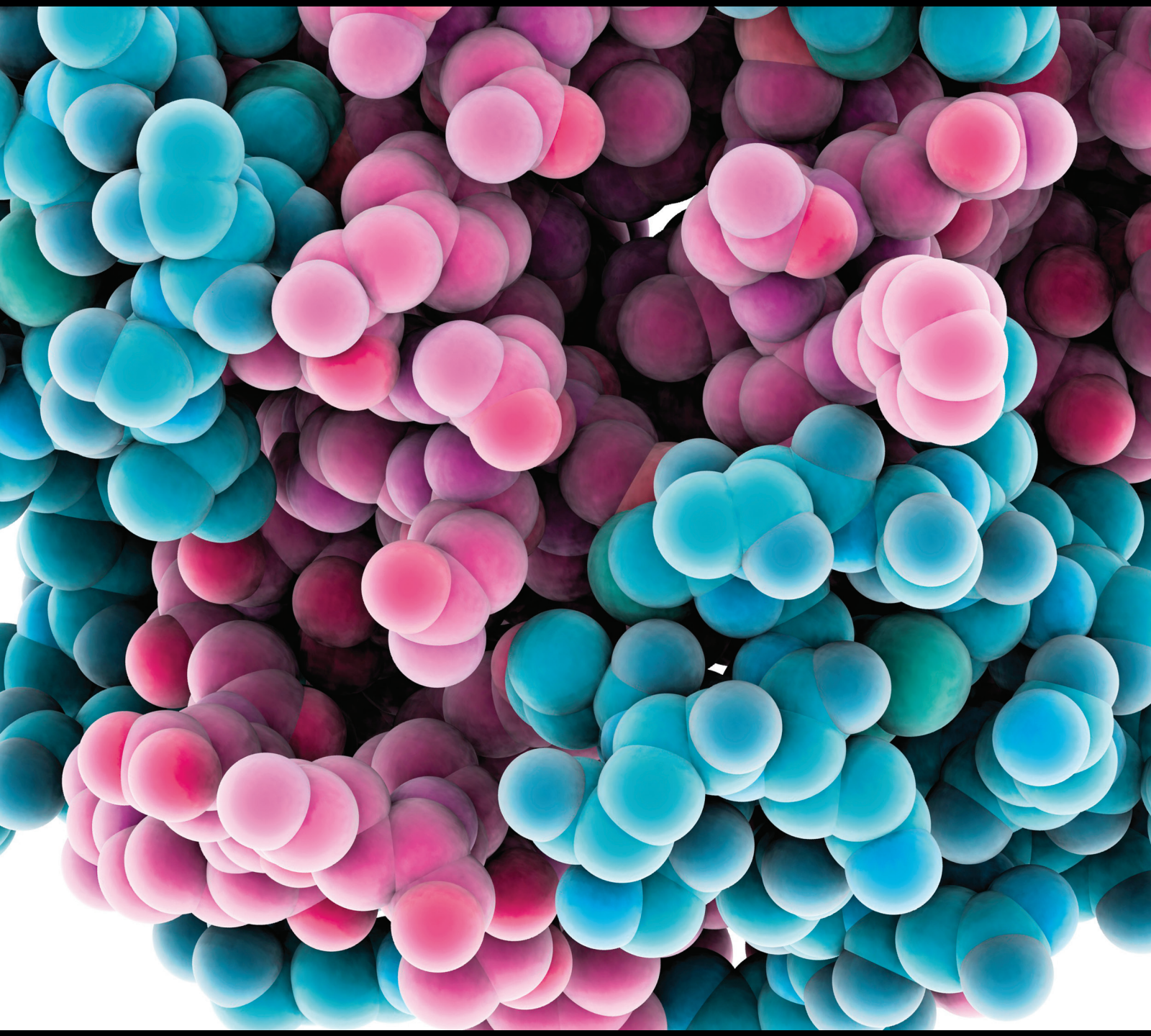
Journal of Diabetes Research

Beta-Cell Function and Its Underlying Mechanism 2015

Guest Editors: Yanbing Li, Chen Wang, Bilal Omar, and Li Chen



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Editorial Board

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Editorial

Beta-Cell Function and Its Underlying Mechanism 2015

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Dynamic changes in beta-cell function during the development and progression of diabetes mellitus are well characterized. However, the driving force and underlying mechanism are poorly understood. In this special issue, we selected multiple original articles and one review which are aiming to explore the beta-cell function and its underlying mechanism from clinical and basic research aspects.

X. Hou et al. investigated β-cell function in 818 newly diagnosed drug naive type 2 diabetic patients grouped by HbA1c values (≤6.5%, 6.5–7%, 7-8%, 8-9%, and ≥9%). They showed that individuals with HbA1c of 6.5–7% exhibited increased HOMA-β as compared to those ≤6.5%. However, the indices decreased substantially with further increment of HbA1c levels. Betatrophin and irisin are two recently identified hormones which may participate in regulating pancreatic β-cell function. L. Wang et al. compared the two hormone levels in 20 NGT and 120 type 2 diabetic patients. Though betatrophin was found significantly elevated, while irisin levels significantly decreased in patients with type 2 diabetes, partial correlation analysis failed to show a correlation between either betatrophin or irisin level and those of β-cell function-related variables.

Particular experience using short-term intensive insulin treatment as the initial management in newly diagnosed type 2 diabetic patients was shared in this issue. J. Ma et al. used intravenous insulin infusion prior to subcutaneous insulin pump therapy in 65 type 2 diabetic patients with HbA1c ≥ 11.80%. They showed that total daily insulin dose in pump therapy was determined by the change of blood glucose in response to intravenous insulin infusion. In another paper, W. Ke et al. randomized 39 newly diagnosed type 2 diabetic patients into either short-term CSII alone or in combination with liraglutide treatment for 12 weeks. They showed that patients with combination therapy achieved euglycemia in shorter time, and their increment of AIR was significantly higher. However, after stopping liraglutide, its effect on beta-cell function disappeared completely.

The basic research papers are interesting. F. Li et al. isolated ISCs from islets of Goto-Kakizaki rats, determined the gene profiles, and assessed their effects on beta-cell function and survival. They concluded that ISCs presented in fibrotic islet of GK rats might be special PSCs, which impaired beta-cell function and proliferation and increased beta-cell apoptosis. Islet brain 1 (IB1) is a candidate gene for diabetes that is required for beta-cell survival and glucose-induced insulin secretion (GSIS). S. Brajkovic et al. showed that chronic exposure of MIN6 cells and isolated rat islets cells to palmitate led to reduction of IB1 mRNA and protein content. Suppression of IB1 level mimicked the harmful effects of palmitate on the beta-cell survival and GSIS. Conversely, ectopic expression of IB1 counteracted the deleterious effects of palmitate on the beta-cell survival and insulin secretion. The lipid droplet-associated proteins FSP27/CIDEC and LSDP5 are associated with hepatic steatosis and insulin sensitivity. Y. Zhu et al. showed that fenofibrate treatment decreased hepatic triglyceride content and FSP27/CIDEC protein expression in mice fed with an HF diet. In contrast, LSDP5 was highly expressed in humans, with elevated expression observed in the fatty liver.

In a review article, X. Luo et al. reviewed the roles of pyruvate, NADH, and complex I in insulin secretion. They proposed that complex I played a crucial role in

the pathogenesis of β cell dysfunction in the diabetic pancreas, based on the fact that chronic hyperglycemia overloads complex I with NADH and leads to enhanced complex I production of reactive oxygen species (ROS), which have been implicated in the pathogenesis of diabetic hyperglycemia.

The articles in this issue bring about some new perspectives that add to our knowledge about beta-cell function and its underlying mechanism. We hope that our readers will be enjoying them.

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Research Article

Modulation Effect of Peroxisome Proliferator-Activated Receptor Agonists on Lipid Droplet Proteins in Liver

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Peroxisome proliferator-activated receptor (PPAR) agonists are used for treating hyperglycemia and type 2 diabetes. However, the mechanism of action of these agonists is still under investigation. The lipid droplet-associated proteins FSP27/CIDEC and LSDP5, regulated directly by PPARγ and PPARα, are associated with hepatic steatosis and insulin sensitivity. Here, we evaluated the expression levels of FSP27/CIDEC and LSDP5 and the regulation of these proteins by consumption of a high-fat diet (HFD) or administration of PPAR agonists. Mice with diet-induced obesity were treated with the PPARγ or PPARα agonist, pioglitazone or fenofibrate, respectively. Liver tissues from *db/db* diabetic mice and human were also collected. Interestingly, FSP27/CIEDC was expressed in mouse and human livers and was upregulated in obese C57BL/6J mice. Fenofibrate treatment decreased hepatic triglyceride (TG) content and FSP27/CIDEC protein expression in mice fed an HFD diet. In mice, LSDP5 was not detected, even in the context of insulin resistance or treatment with PPAR agonists. However, LSDP5 was highly expressed in humans, with elevated expression observed in the fatty liver. We concluded that fenofibrate greatly decreased hepatic TG content and FSP27/CIDEC protein expression in mice fed an HFD, suggesting a potential regulatory role for fenofibrate in the amelioration of hepatic steatosis.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), one of the most common liver diseases worldwide, encompasses a spectrum of liver conditions, ranging from simple steatosis, also called simple fatty liver (SFL), to nonalcoholic steatohepatitis (NASH), advanced fibrosis, and cirrhosis [1]. NAFLD has become a major health concern, with as many as 20%–40% of the general population in western countries and 5%–40% of the general population in countries in the Asia-Pacific region affected by NAFLD [2, 3]. Patients with NAFLD are at significantly higher risk for the development of type 2 diabetes (T2D) and cardiovascular disease [4]. Thus, unraveling the pathogenesis of NAFLD and investigating effective treatment options are essential.

As the most benign form of NAFLD, SFL is characterized by excessive lipid accumulation, mainly in the form of lipid droplets (LDs) in hepatocytes. Structurally, LDs consist of a neutral lipid core surrounded by a phospholipid monolayer and proteins embedded in or bound to the phospholipid layer, namely, LD-associated proteins (LDAPs). Importantly, LDs are now recognized not merely as a static neutral lipid storage site but instead as multifunctional organelles involved in lipid metabolism and transport, intracellular trafficking, signaling, and cytoskeletal organization [5]. LDAPs are crucial for LD formation, growth, transport, and hydrolysis and play key roles in various functions of LDs [6]. Most importantly, increasing evidence has shown that there is a relationship between LDAPs and lipid metabolism in hepatocytes of rodents and humans [7]. Fat-specific protein 27 (FSP27)/cell death-inducing DFF45-like effector-C (CIDEC) and lipid storage droplet protein 5 (LSDP5), two members of the LDAP family of proteins, have been shown

to facilitate liver steatosis and regulate insulin sensitivity [8, 9]. Overexpression of FSP27/CIDEC in hepatocytes leads to increased hepatic triglyceride (TG) levels [8], whereas knockout of FSP27/CIDEC in mice induces lean phenotypes [10]. Moreover, FSP27/CIDEC-null mice are resistant to diet-induced obesity and insulin resistance [10], and exposure of primary rat hepatocytes to free fatty acids (FFAs) increases LSDP5 expression and lipid accumulation [9]. Both FSP27/CIDEC and LSDP5 are positively regulated by peroxisome proliferator-activated receptor (PPAR); specifically, LSDP5 is regulated by PPARα, and FSP27/CIDEC is regulated by PPARγ [8, 11, 12]. Thus, activation of either PPARγ or PPARα may lead to upregulation of FSP27/CIDEC or LSDP5, thereby increasing lipid accumulation. Fenofibrate, a PPARα agonist, and pioglitazone, a PPARγ agonist, are widely used in the clinical setting for the management of dyslipidemia and insulin resistance. It is not known whether PPAR activation (fenofibrate and pioglitazone treatment) will increase hepatic lipid content by inducing LADPs expression. If it is true, it would ultimately impair the role of PPAR activators on insulin-sensitizing effects.

In the present study, we used a mouse model of SFL induced by high-fat diet (HFD) to measure the expression of FSP27/CIDEC and LSDP5 in the liver. The expression of FSP27/CIDEC and LSDP5 in liver tissue sample from human with fatty liver was also studied. Moreover, we investigated the effects of PPAR activation on the regulation of these LADPs in HFD-induced obese mice treated with fenofibrate or pioglitazone for 20 weeks. Lastly, the effects of PPAR activators on glucose/lipid metabolism and insulin resistance in HFD mice were also determined.

2. Materials and Methods

2.1. Animal Study. Male C57BL/6J mice (4 weeks old) were purchased from Shanghai SLAC Laboratory Animal Co. Ltd., China (certificate number: SCXK [Shanghai] 2003-0003), and were housed in rooms with a 12-hour light/dark cycle (lights on 07:00 h). Prior to the dietary and drug manipulation, all mice were provided with standard chow (Shanghai Laboratory Animal Center (SLAC): 55% of energy as carbohydrates, 21% as protein, and 14% as fat) and water *ad libitum*. After 1 week of acclimation, the animals were randomly assigned to receive one of the following treatments for 20 weeks: chow diet, HFD (20% of energy as carbohydrates, 20% as protein, and 60% as fat, as a percentage of total kcal, manufactured by SLAC), HFD + fenofibrate (30 mg/kg body weight, Sigma-Aldrich, St. Louis, MO, USA), and HFD + pioglitazone (10 mg/kg body weight, Sigma-Aldrich). Drugs were administered through a feeding needle once per day; the doses were chosen according to previous studies in which similar doses were used for metabolic studies in mice [13, 14]. Male diabetic C57BL/KsJ *db/db* mice (6 weeks old) were fed a standard chow diet for 12 weeks until they developed spontaneous diabetes. Body weights were measured daily for all mice, and fasting blood samples were collected from the tail vein every 4 weeks. At the end of the study, all animals were fasted for 2 h prior to euthanasia inhalation of isoflurane (Abbott Laboratories, Abbott Park, IL, USA), and livers were surgically collected, frozen in liquid nitrogen, and stored at −80°C for further studies. Adipose tissues were also collected and weighed.

2.2. Intraperitoneal Glucose Tolerance Test and Insulin Tolerance Test. After a 1-week acclimation, male C57BL/6J mice were fasted for 10 h before the intraperitoneal glucose tolerance test (GTT) at week 19. After a sample of fasted blood was collected from tail bleeding, animals were given glucose (1 g/kg body weight) by intraperitoneal injection. Blood glucose readings were taken using a glucometer (Freestyle Freedom, Abbott Laboratories) at 0, 30, 60, and 120 min after injection. Insulin tolerance tests (ITT) were carried out 1 week after GTTs. Neutral insulin (1 U/kg body weight, Novo Nordisk, Denmark) was injected intraperitoneally after a 6-hour fast. Blood glucose levels were measured at 0, 15, 30, 60, 90, and 120 min after injection. The areas under the curves for blood glucose in GTT (AUC_g) and ITT (AUC_{itt}) were calculated.

2.3. Blood Biochemistry. Serum biochemistry parameters, including triglycerides (TGs), total cholesterol (TC), high-density lipoprotein- (HDL-) cholesterol (HDL-C), and low-density lipoprotein- (LDL-) cholesterol (LDL-C), were measured after overnight fasting in mice. The measurements were performed with a parallel, multichannel analyzer (Glamour 2000, MD Inc., Silicon Valley, California, USA). Serum insulin was determined manually using an enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Sweden).

2.4. Human Liver Tissues Collection. The liver tissue samples were collected at the Department of General Surgery (Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China) from man subjects undergoing resection of benign focal hepatic lesions. Samples with hepatitis, cirrhosis, or chronic alcohol use were excluded. The samples were immediately shock-frozen and stored at −80°C. All tissues had been examined by a pathologist who was blinded to the study design. Liver tissues with less than 5% hepatic steatosis were classified into the nonfatty liver (Non-FL) group, while those with more than 20% hepatic steatosis were classified into the fatty liver (FL) group. The clinical background data of both groups were presented in Supplementary Table S1 (see Supplementary Material available online at http://dx.doi.org/10.1155/2016/8315454). The study was approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital, following the principles of the Declaration of Helsinki. Written informed consent was obtained from each patient.

2.5. Analysis of TG Levels in the Liver. For TG measurements, 50 mg liver tissue was homogenized in standard phosphate-buffered saline. Lipids were extracted using a heptane-isopropanol-Tween solution [15]. TG concentrations were measured manually by the enzymatic GPO-PAP method using commercial kits (KHB Instruments, China) and were normalized to sample weight for accurate quantification.

Table 1: Metabolic phenotypes in mice before and after treatment.

Variables	Time (week)	Chow	HF	HF-P	HF-F	db/db
Body weight (g)	0	16.3 ± 1.0	17.6 ± 1.2	15.1 ± 0.3	14.6 ± 0.3	$20.9 \pm 0.8^{a*b*}$
	8	24.4 ± 0.9	$28.4 \pm 0.4^{a**}$	$25.5 \pm 0.2^{b*}$	$24.3 \pm 1.1^{b*}$	$49.9 \pm 1.2^{a**b**}$
	12	24.2 ± 0.6	$27.2 \pm 1.4^{a*}$	26.5 ± 0.8	25.0 ± 0.9	$57.6 \pm 3.2^{a**b**}$
	16	23.1 ± 1.3	$28.9 \pm 1.3^{a**}$	29.2 ± 0.7	26.5 ± 0.6	
	20	24.4 ± 0.9	$28.4 \pm 1.2^{a**}$	29.0 ± 0.8	25.8 ± 0.8	
Fasting glucose (mmol/L)	0	6.1 ± 0.2	6.2 ± 0.2	6.6 ± 0.1	6.6 ± 0.1	$8.2 \pm 0.3^{a*b*}$
	8	6.2 ± 0.4	$8.4 \pm 0.4^{a**}$	8.1 ± 0.5	7.9 ± 0.3	$15.8 \pm 2.2^{a**b**}$
	12	5.8 ± 0.4	$8.4 \pm 0.7^{a**}$	$6.1 \pm 0.3^{b**}$	$6.1 \pm 0.3^{b**}$	$11.3 \pm 1.7^{a**b**}$
	16	5.4 ± 0.2	$8.0 \pm 1.4^{a*}$	6.1 ± 0.2	5.8 ± 0.2	
	20	6.1 ± 0.5	$10.7 \pm 0.4^{a**}$	9.2 ± 0.5	$8.1 \pm 0.6^{b**}$	
Epididymal fat (g)	20 (12†)	0.32 ± 0.05	$0.71 \pm 0.10^{a**}$	0.72 ± 0.05	$0.37 \pm 0.05^{b**}$	2.15 ± 0.36
Subcutaneous fat (g)	20 (12†)	0.23 ± 0.03	$0.53 \pm 0.09^{a**}$	0.78 ± 0.03	$0.25 \pm 0.03^{b**}$	10.24 ± 1.63
Fasting serum insulin (ng/mL)	20 (12†)	0.78 ± 0.04	$1.78 \pm 0.23^{a**}$	$0.91 \pm 0.14^{b*}$	$1.04 \pm 0.14^{b*}$	4.07 ± 0.77
Serum TG (mmol/L)	20 (12†)	1.06 ± 0.15	0.92 ± 0.14	1.24 ± 0.09	1.15 ± 0.11	1.54 ± 0.15
Serum TC (mmol/L)	20 (12†)	0.77 ± 0.08	$1.48 \pm 0.14^{a**}$	$0.97 \pm 0.05^{b**}$	$1.08 \pm 0.06^{b**}$	1.89 ± 0.13
Liver TG (μmol/g)	20 (12†)	7.0 ± 0.5	$12.3 \pm 0.7^{a**}$	10.9 ± 1.1	$7.5 \pm 0.8^{b**}$	17.3 ± 3.0
Liver TC (μmol/g)	20 (12†)	6.0 ± 0.4	9.3 ± 1.4	7.2 ± 0.5	6.9 ± 0.7	14.6 ± 0.3

Data represent means ± SEM. Statistical significance of differences between groups was analyzed with one-way analysis of variance (ANOVA) followed by Bonferroni's test.

Chow: mice fed a standard chow diet; HF: mice fed a high-fat diet; HF-P: mice fed a high-fat diet and treated with pioglitazone; HF-F: mice fed a high-fat diet and treated with fenofibrate; TC: total cholesterol; TG: triglyceride.

†Time for db/db. [a]Versus Chow, [b]Versus HF; $^{*}P < 0.05$, $^{**}P < 0.01$; $n = 7$.

2.6. Western Blotting. Liver tissues (50 mg) were ground into a powder under liquid nitrogen, and hepatic protein was extracted by incubation at 4°C in lysis buffer containing protease inhibitors, followed by sonication. The lysates were centrifuged at 12,000 rpm for 20 min, and the supernatants were collected. Protein concentrations were determined by the bicinchoninic acid method. After heating to 95°C for 5 min, the proteins were size-fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels and then transferred to polyvinylidene difluoride membranes (Millipore, MA, USA) at 100 V for 70 min. After washing three times with Tris-buffered saline (TBS), the membranes were blocked with 5% dried nonfat milk (Nestle, China) in TBS-Tween 20 (TBS-T, pH 7.4) and then incubated with appropriate primary antibodies targeting FSP27/CIDEC (PAI-4316, diluted 1 : 1,000, Thermo Fisher Scientific, Loughborough, UK) or LSDP5 (ab63970, diluted 1 : 1,000, Abcam, MA, USA) overnight at 4°C. The membranes were then washed three times with TBS-T for 10 min each and incubated with horseradish peroxidase-labeled donkey anti-rabbit IgG (sc-2313, diluted 1 : 5,000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) in TBS-T for 1 h at room temperature. Membranes were then washed three times for 10 min each in TBS-T and visualized using the enhanced chemiluminescence method (Thermo Scientific Pierce, Rockford, IL, USA). Protein expression values were standardized against β-actin protein expression (4697s, Cell Signaling Technology, Danvers, MA, USA). The average intensity for each band was quantified with Image J (NIH, Bethesda, MD, USA).

2.7. Statistical Analysis. All data are represented as means ± SEM. For statistical analysis, the differences between groups were examined with one-way analysis of variance followed by Bonferroni's test using SPSS 11.0, and differences with two-tailed P values less than 0.05 were considered statistically significant.

3. Results

3.1. Metabolic Changes Associated with HFD-Induced Obesity in Mice. After 20 weeks of consuming an HFD, mice became obese, exhibiting marked increases in body weight, fat mass, glucose level, and TG content in the liver although they did not develop overt diabetes as db/db mice in week 12 (Table 1). In addition, significant changes in serum lipids were observed, as shown by increases in total cholesterol levels (0.77 ± 0.08 versus 1.48 ± 0.14 mM, $P < 0.01$). Mice became profoundly hyperinsulinemic and insulin intolerant, suggesting the acquisition of insulin resistance (Table 1 and Figures 1(b) and 1(d)), and were clearly glucose intolerant (Figures 1(a) and 1(c)).

3.2. Effects of PPAR Agonists on HFD-Induced Obesity in Mice. In order to determine the effects of an HFD and treatment with PPAR agonists on metabolism, C57BL/6J mice were treated as described in Section 2, and metabolic parameters were measured. Fenofibrate treatment greatly inhibited the increase in body weight and fat mass (epididymal fat and subcutaneous fat) induced by consumption of an HFD. Fasting glucose levels were reduced early in the experimental

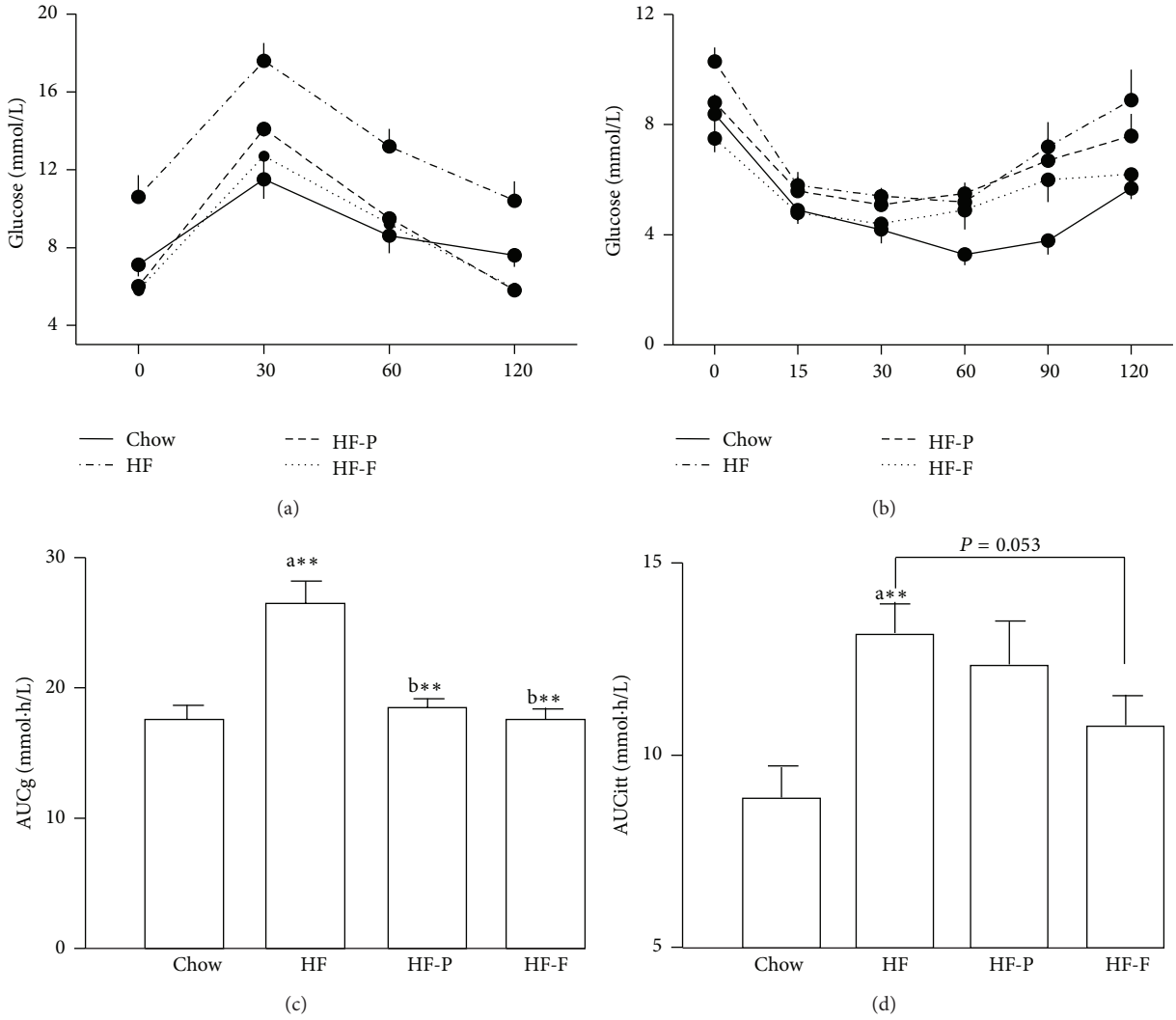

Figure 1: PPAR agonists' treatment improved glucose tolerance and insulin sensitivity in high-fat diet-induced obese mice. Mice were fed with either a normal chow diet (Chow) or a high-fat diet (HF) for 20 weeks and treated with either pioglitazone or fenofibrate. (a) Intraperitoneal glucose tolerance test (GTT), (b) insulin tolerance test (ITT), (c) AUC of GTT, and (d) AUC of ITT. HF-P: mice fed a high-fat diet and treated with pioglitazone; HF-F: mice fed a high-fat diet and treated with fenofibrate. a: versus Chow and b: versus HF; $^{**}P < 0.01$; $n = 7$.

period in mice treated with either fenofibrate or pioglitazone. Dyslipidemia was also improved significantly by both PPAR agonists (Table 1). Furthermore, both fenofibrate and pioglitazone corrected glucose tolerance and insulin sensitivity (Figures 1(a)–1(d)). Most importantly, TG levels were reduced in the livers of fenofibrate-treated mice fed an HFD compared with untreated mice fed an HFD (7.5 ± 0.8 versus 12.3 ± 0.7 μmol/g, $P < 0.01$, Table 1).

3.3. FSP27/CIDEC and LSDP5 Protein Expression in Liver from HFD-Induced Obese Mice Treated with PPAR Agonists. We further investigated the effects of PPAR activators on the expression of LDAPs. Our results suggested that hepatic FSP27/CIDEC protein expression was significantly enhanced by consumption of an HFD in mice. Compared with mice consuming the standard chow diet, mice fed an HFD exhibited an average 97% increase in FSP27/CIDEC expression ($P < 0.05$, Figure 2(a)). The expected increase in FSP27/CIDEC expression was also observed in the livers of *db/db* diabetic mice, although this difference was not significant (Figure 2(a)). Moreover, after treatment with fenofibrate or pioglitazone daily for 20 weeks, FSP27/CIEDC expression was reduced by 51% and 29%, respectively; this difference was statistically significant for fenofibrate only ($P < 0.05$, Figure 2(b)). Interestingly, LSDP5 expression was nearly undetectable in the livers of C57BL/6J and *db/db* diabetic mice (Supplementary Figure S1).

3.4. Expression of FSP27/CIDEC and LSDP5 in Liver Sample from Subjects with Fatty Liver. In order to validate the association of LDAP expression and hepatic steatosis observed in animal models, we further measured the protein expression of FSP27/CIDEC and LSDP5 in human livers. FSP27/CIDEC expression tended to increase in patients in the FL group

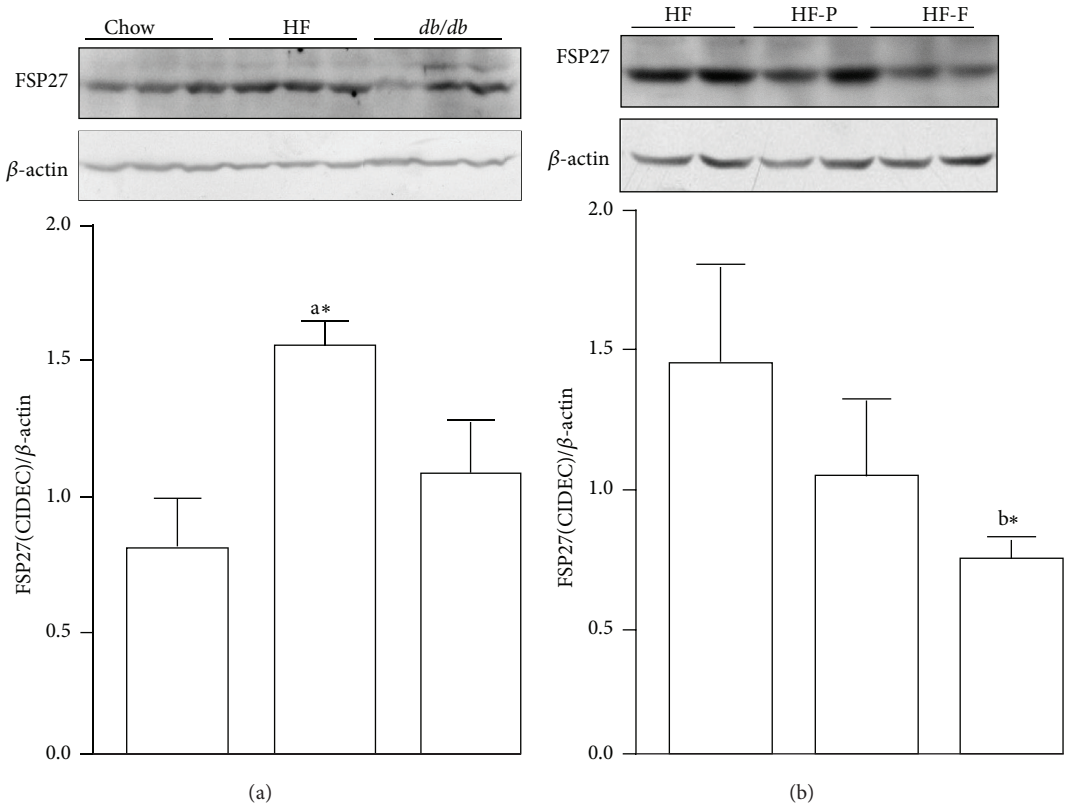

Figure 2: FSP27/CIDEC protein is highly expressed in mouse fatty liver. Immunoblot analyses of FSP27/CIDEC protein abundance in liver lysates (upper panel). Quantification of the protein levels was normalized to β-actin (lower panel). (a) FSP27/CIDEC protein level in liver from C57BL/6 mice fed with a normal chow diet (Chow) or high-fat diet (HF) for 20 weeks or from db/db mice. (b) FSP27/CIDEC protein level in liver from mice fed with HF diet and treated with pioglitazone or fenofibrate. Data are the mean ± SEM (n = 4–7). a: versus Chow and b: versus HF; $^*P < 0.05$. HF-P: mice fed an HF diet and treated with pioglitazone; HF-F: mice fed an HF diet and treated with fenofibrate.

compared with patients in the Non-FL group (Figure 3(a)) ($P = 0.08$). Surprisingly, the expression of LSDP5 was significantly higher in patients in the FL group compared with patients in the Non-FL group (1.59 ± 0.15 versus 0.90 ± 0.12, resp., $P < 0.05$, Figure 3(b)).

4. Discussion

In the present study, we provided evidence supporting previous findings that both fenofibrate (a PPARα agonist) and pioglitazone (a PPARγ agonist) significantly improve insulin sensitivity and glucose tolerance, with fenofibrate exhibiting superior effects in mice fed an HFD. More importantly, we found for the first time that fenofibrate treatment in mice fed an HFD resulted in a significant decrease in hepatic lipid content, accompanied by reduced FSP27/CIDEC protein expression in the liver. Furthermore, LSDP5 was markedly elevated in liver tissues from patients with FL. These findings indicated that fenofibrate or pioglitazone had no side effects on LSDP5 and FSP27/CIDEC protein expression. In contrast, FSP27/CIDEC may be associated with amelioration of steatohepatitis in the context of long-term fenofibrate treatment. In humans, LSDP5 was positively correlated with hepatic lipid content, consistent with the observations in rodent models.

FSP27/CIDEC belongs to the CIDE family, which includes CIDEA, CIDEB, and CIDEC. All of these proteins contain a conserved CIDE-N domain [15]. CIDEC is the human homolog of mouse FSP27 [16]. CIDE proteins are important regulators of energy homeostasis and are closely linked to the development of metabolic disorders [15]. As a member of the CIDE family, FSP27/CIDEC plays important roles in hepatic steatosis [8, 11, 17, 18]. FSP27/CIDEC is dramatically upregulated in the livers of *ob/ob* and mice with HFD-induced obesity [8, 19]. In vitro and in vivo studies have shown that forced expression of FSP27/CIDEC in hepatocytes leads to an increase in the content of hepatic TGs [8]. Targeted knockdown of FSP27/CIDEC expression in the livers of *ob/ob* mice partially ameliorates FL pathology [8]. Moreover, liver sections from mice injected with adenovirus expressing FSP27 shRNA exhibit smaller and less numerous LDs as compared to mice injected with adenovirus expressing scramble shRNA [8]. Our finding that FSP27/CIDEC was upregulated in FLs from mice fed an HFD was in agreement with the results of a previous report [19]. Moreover, we observed similar results in humans, consistent with a study in patients who underwent gastric bypass surgery, in which hepatic *CIDEC* was significantly downregulated and symptoms of steatosis were ameliorated due to weight loss at 1 year after surgery [17].

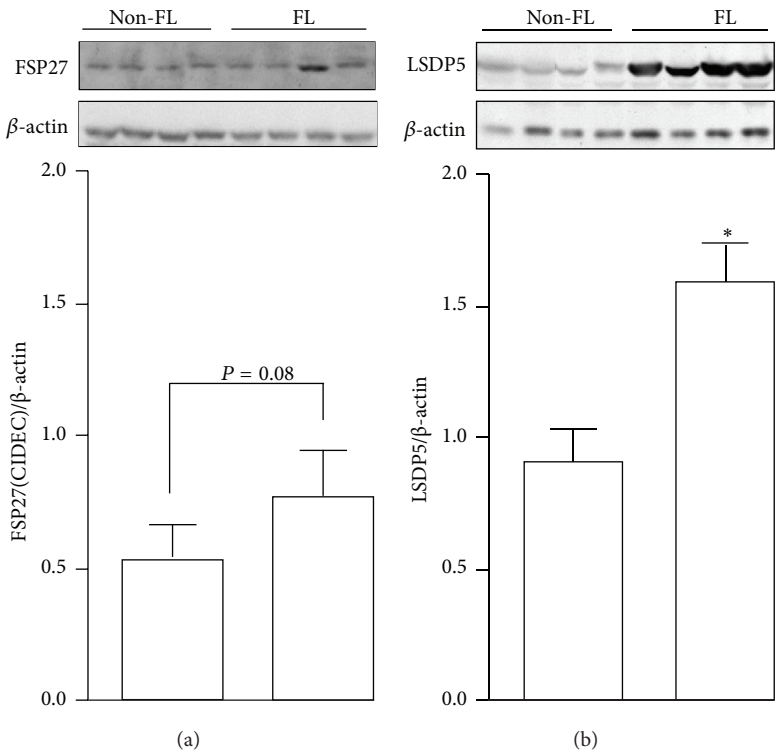

Figure 3: FSP27/CIDEC and LSDP5 protein expression increased in human fatty liver. Immunoblot analyses of FSP27/CIDEC (a) and LSDP5 (b) protein expression (upper panel) from human liver tissue samples with or without fatty liver. Quantification of the protein levels was normalized to β-actin (lower panel). Data are the mean ± SEM ($n = 4$). $^{*}P < 0.05$. Non-FL: subjects without fatty liver and FL: subjects with fatty liver.

FSP27/CIDEC is induced by PPARγ activators and functions as a direct downstream target of hepatic PPARγ [8]. Using a subtractive cloning strategy, researchers have shown that *FSP27* cDNA is specifically expressed in FLs of rosiglitazone-treated liver-specific PPARγ-null (*ob*/*ob*-PPARγ/C^{+}) mice [8]. Additionally, PPARγ is elevated in FLs from murine model of diabetes and obesity [20, 21] and is critical for the development of hepatic steatosis [22, 23]. PPARγ deficiency in the livers of *ob*/*ob* mice and mice with HFD-induced obesity dramatically improves hepatic steatosis. Indeed, some studies have reported that prolonged treatment of obese and diabetic mice with thiazolidinediones (TZDs, selective PPARγ ligands and activators), including troglitazone, rosiglitazone, and pioglitazone, results in the development of severe hepatic steatosis [24]. Since FSP27/CIDEC is involved in PPARγ-dependent hepatic steatosis [8], it is therefore necessary to determine whether the effects of PPARγ agonists on the formation of FL were mediated by activation of FSP27/CIDEC expression. PPARγ agonists, including troglitazone, rosiglitazone, and pioglitazone, are widely used as insulin sensitizers in the clinical setting. However, troglitazone and rosiglitazone have been withdrawn from the market because of their significant side effects; therefore, only pioglitazone is currently available for clinical use in humans. To our surprise, in the present study, treatment with pioglitazone for 20 weeks neither significantly induced the expression of FSP27/CIDEC nor increased hepatic lipid content in mice with HFD-induced obesity, which was in agreement with a study of alcohol-induced FL [19]. However, Satoh et al. reported that treatment with pioglitazone (9 mg/kg) for 6 weeks exacerbated hepatic steatosis and markedly elevated FSP27 expression in ddY-H mice (a model of spontaneous insulin resistance) fed standard chow [25]. This discrepancy may be explained as follows. First, pioglitazone treatment and consumption of an HFD both activate PPARγ in the liver [26, 27]. Therefore, the induction of FSP27/CIDEC expression by PPARγ may not differ substantially following pioglitazone treatment in mice fed an HFD. Second, given the positive correlation between hepatic TG content and FSP27/CIDEC expression in mice with HFD-induced obesity [19], hepatic FSP27/CIDEC levels may be similar because of the similar hepatic TG contents in mice treated with or without pioglitazone. Third, these studies used different mouse models, which could lead to differences in response to treatment. In the present study, pioglitazone treatment for 20 weeks did not significantly affect hepatic lipid content in mice fed an HFD. However, because these results differ from previous works [28–30], the effects of long-term pioglitazone treatment on hepatic TG content are still unclear. Thus, while it is evident that different models of obesity and diabetes may yield different results, our data from mice fed an HFD supported that long-term pioglitazone treatment may not increase hepatic lipid content in this animal model.

Factors other than PPARγ may also play a role in the regulation of FSP27/CIDEC. Data from our animal research

suggested that PPARα may also regulate the expression of FSP27/CIDEC as long-term treatment with fenofibrate, a PPARα agonist, decreased hepatic FSP27/CIDEC expression and lipid content. Fenofibrate is widely used for the treatment of hypertriacylglycerolemia in patients with T2D. Although conflicting results have been reported in human studies [31, 32], data from our study and other studies have shown that fenofibrate treatment in rodents improved insulin resistance and fat deposition in the liver [33, 34]. As the major target tissue of fenofibrate activity, the liver may be responsible for the improved insulin sensitivity observed in fenofibrate-treated animals exhibiting lipid accumulation. As shown in our study and other studies, fenofibrate prevented HFD-induced hepatic TG accumulation and insulin resistance [35, 36]. However, the detailed mechanisms remained largely unknown. Our finding of fenofibrate-induced FSP27/CIDEC downregulation suggested a new mechanism for FSP27/CIDEC regulation and improved our understanding of the mechanism of PPARα-dependent improvement in symptoms of FL. However, the negative regulation of FSP27/CIDEC by fenofibrate may be indirect as the PPARα agonist Wy-14643 did not activate the functional peroxisome proliferator-activated receptor response element (PPRE) in the mouse *fsp27* promoter in HEK293 cells [8]. Moreover, forced expression of FSP27/CIDEC in hepatocytes significantly decreases the mitochondrial β-oxidation, which is negatively associated with TG accumulation in the liver [8]. Additionally, fenofibrate induces hepatic mitochondrial β-oxidation in obese rats [11]. These data suggest that fenofibrate increases hepatic mitochondrial β-oxidation by indirectly downregulating the expression of FSP27/CIDEC and ultimately decreasing hepatic lipid content. Therefore, it will be interesting to investigate the effects of fenofibrate on mitochondrial β-oxidation and lipid accumulation in the livers of liver-specific FSP27/CIDEC-knockout mice. During the preparation of this paper, a study published in *Hepatology* showed that the promoter of *FSP27*β (the major isoform in the liver) was activated by the liver-enriched transcription factor cyclic AMP-responsive element-binding protein H (CREBH) [18]. The *CREBH* promoter contains a functional PPPE site, which interacts with PPARα [37]. CREBH physically interacts with PPARα and regulates a variety of genes involved in fatty acid oxidation and hepatic lipid accumulation [38, 39]. Thus, further studies are required to investigate whether FSP27/CIDEC is involved in the interaction networks of CREBH and PPARα in the liver.

In the present study, we observed for the first time that the expression of LSDP5 protein was markedly upregulated in FLs compared with normal livers in humans. LSDP5 is a newly identified member of the perilipin, ADFP, and TIP47 (PAT) family, which is ubiquitously expressed in tissues that exhibit high levels of fatty acid oxidation, including the heart, muscle, and liver [12, 40, 41]. In hepatocytes, LSDP5 is thought to contribute to TG accumulation by negatively regulating lipolysis and fatty acid oxidation [9]. However, the role of LSDP5 in hepatic steatosis in vivo is largely unknown. LSDP5 mRNA and/or protein levels are upregulated in different animal models with FL (e.g., rat fed an HFD, mice with FL dystrophy, and mice with β3 adrenergic receptor agonist-induced acute hepatic steatosis) [42–44]. Until now, no studies had described the hepatic expression of LSDP5 in humans. While our data support the hypothesis that LSDP5 protein expression is correlated with intrahepatic fat content in human subjects, more work is needed to confirm the association in a large sample population and to characterize its physiological and pathological roles in human hepatic lipid metabolism in future studies.

LSDP5 is modulated by both PPARα and fasting in the mouse liver; thus, we investigated the regulation of LSDP5 protein expression by consumption of an HFD and treatment with PPAR (PPARα and PPARγ) agonists in mice. To our surprise, our data showed that LSDP5 expression was barely detectable in the livers of these mice, and neither HFD nor PPAR agonists induced the expression of this protein. This finding was somewhat inconsistent with other studies, in which LSDP5 protein was expressed in mouse liver [40, 43, 44]. The exact reasons for the discrepancy between our study and others are unknown. One possible explanation for the difference may result from the samples taken from the mice at different physiological conditions, in which our liver samples were obtained from fed mice, whereas other studies may be from fasted mice [43, 44]. Both PPARα and fasting was reported to regulate the expression of LSDP5 in liver, and fasting statues itself induced LSDP5 mRNA expression independent of PPARα activation in mouse liver [40]. Our data in the present study suggested that fasting/feeding might be a stronger factor for the expression level of LSDP5 than PPARα in vivo. Future study might be needed to explore the possibility. Moreover, different animal models used in the studies may be another possibility for the differences [42–44].

In conclusion, we reported in the present study that the hepatic expression of FSP27/CIDEC and LSDP5 was upregulated in humans and that FSP27/CIDEC was increased in FLs of mice following consumption of an HFD. Long-term fenofibrate treatment decreased FSP27/CIDEC expression and hepatic TG content in mice fed an HFD. Our data suggest a potential new mechanism for FSP27/CIDEC expression in the liver and indicate a novel molecular mechanism of action of PPAR agonists, which should be further investigated.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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Research Article

Islet Stellate Cells Isolated from Fibrotic Islet of Goto-Kakizaki Rats Affect Biological Behavior of Beta-Cell

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We previously isolated islet stellate cells (ISCs) from healthy Wistar rat islets. In the present study, we isolated "already primed by diabetic environment" ISCs from islets of Goto-Kakizaki rats, determined the gene profile of these cells, and assessed the effects of these ISCs on beta-cell function and survival. We detected gene expression of ISCs by digital gene expression. INS-1 cell proliferation, apoptosis, and insulin production were measured after being treated with ISCs supernatant (SN). We observed the similar expression pattern of ISCs and PSCs, but 1067 differentially expressed genes. Insulin production in INS-1 cells cultured with ISC-SN was significantly reduced. The 5-ethynyl-2′-deoxyuridine-positive INS-1 cells treated with ISC-SN were decreased. Propidium iodide- (PI-) positive INS-1 cells were 2.6-fold higher than those in control groups. Caspase-3 activity was increased. In conclusion, ISCs presented in fibrotic islet of GK rats might be special PSCs, which impaired beta-cell function and proliferation and increased beta-cell apoptosis.

1. Introduction

Type 2 diabetes mellitus (T2DM) has reached pandemic proportions, and current predictions show that this trend will continue [1, 2]. Therefore, achieving a better understanding of this complex disease is imperative. Islet fibrosis in T2DM has received increasing scientific attention [3–10]. Studies showed that pancreatic stellate cells (PSCs) have important functions in islet fibrogenesis in both rodent animal models and human patients with T2DM [6, 7, 11, 12]. In addition, we observed that high glucose aggravates the detrimental effects of pancreatic stellate cells on beta-cell function [13]. To elucidate the underlined mechanisms responsible for islet fibrosis in the late stage of T2DM, we find that endocrine pancreatic islets contain cells resembling PSCs and suggest these may contribute to islet fibrosis in T2DM [14].

In the normal pancreas, PSCs are quiescent and are found in low abundance [15, 16]. Upon pancreatic injury or pancreatic inflammation, PSCs lose their vitamin A stores and transform from "activated" into myofibroblast-like phenotypes, which highly proliferate, migrate, synthesize, and secrete excessive amounts of the extracellular matrix (ECM) proteins, resulting in tissue fibrosis [15–23].

The function of PSCs in islet fibrosis has been the subject of several studies for years. *In vivo* studies have shown that PSCs are present in rat islets and are involved in islet fibrogenesis in several animal models of T2DM [10, 11, 24–27]. In humans, PSCs are also present in islets of T2DM patients and possibly have a function in the progression of islet fibrosis [11]. Fibrosis is one of the major factors leading to progressive pancreatic beta-cell loss and dysfunction [6, 11, 28–33]. Efforts have been made for developing antifibrotic strategies to ameliorate islet fibrosis and the progression of T2DM [6, 12, 34–36]. *In vivo* studies showed that the attenuation of PSC activation reduces islet fibrosis [6, 12, 26, 36] or increases insulin content [26].

Kikuta et al. recently reported that indirect coculture of RIN-5F cells with PSCs results in decreased insulin production and increased cell apoptosis [37], and very recently published data showed that activated PSCs can impair pancreatic islet function in mice [38]. We observed that PSCs transplantation exacerbated the impaired β-cell function in GK rats [13] and demonstrated that PSCs resemble cells within endocrine pancreatic islets [14]. However, in T2DM, the PSCs that presented intra-/peri-islets were exposed to the islet niche, such as marked hyperglycemia [28, 39, 40], oxidative stress [41–43], and inflammation [4, 28, 39]. These factors could affect PSCs activation and proliferation and subsequently stimulate the production of endogenous inflammatory mediators in PSCs [17]. Thus, PSCs were already "activated" by the environmental conditions of T2DM *in vivo*. Therefore, we isolated stellate cell from fibrotic islets of Goto-Kakizaki (GK) rats, and our previous data pushed us to question whether these population cells were a special type of PSCs and which effect is on biological behavior of beta-cells.

2. Methods

2.1. Animals and Ethics Statement. Rats were housed in cages (three rats per cage) under a 12 h/12 h light/dark cycle. Rats were given free access to food and water ad libitum. Animal experiments were approved by the Southeast University Animal Care and Use Committee according to institutional guidelines and national animal welfare.

2.2. Isolation of PSCs. PSCs were isolated from 8-week-old Wistar rats as described previously [16]. PSCs were cultured in Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 (1 : 1 v/v) containing 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, USA). Cell purity was assessed by immunostaining for vimentin (100%), α-SMA (>95%), and desmin (20–50%).

2.3. Isolation and Culture of ISCs and Preparation of ISC-SN. Islets were isolated from four-month-old male GK rat pancreas as previously described [4]. In brief, pancreas tissues were digested with collagenase V (1 mg/mL, w/v) (Sigma, St. Louis, MO, USA) at 37°C for 15 min to 18 min. Islets were purified by handpicking twice under a stereomicroscope. Then, islets were precultured in RPMI-1640 supplemented with L-glutamine containing 10% (v/v) fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA) overnight followed by handpicking.

After 48 h in culture, ISCs began to grow out of GK islets. After 5 d, cells were subcultured in DMEM/Ham's F12 (1 : 1, v/v) containing 10% FBS. Cells at passages 3 to 8 were used for experiments.

To prepare ISC-SN, cells grown near confluence were cultured with DMEM/F12 serum-free medium plus 0.2% BSA for another 48 h. The culture medium was collected, centrifuged, filtered, and stored at −80°C until use.

2.4. Immunofluorescence Staining. After shortly being washed with cold phosphate buffer saline (PBS), cells were fixed with 4% paraformaldehyde at room temperature for 20 min and subsequently stained with primary antibodies at the following dilutions: α-SMA, (1 : 100, DAKO, Hamburg, Germany), vimentin (1 : 200, DAKO), and desmin (1 : 50, DAKO), followed by fluorescent secondary antibodies (DAKO). The nuclei were then counterstained with bisbenzimide. Photos of eight different areas in each well were taken using a microscope with 100x magnification. A minimum of 500 cells in each experimental group was analyzed ($n = 3$).

2.5. Digital Gene Expression (DGE) Profile of ISCs and PSCs. The passage 3 ISCs and PSCs were used for DGE analysis. DGE was performed by the BGI Tech (Shenzhen, China).

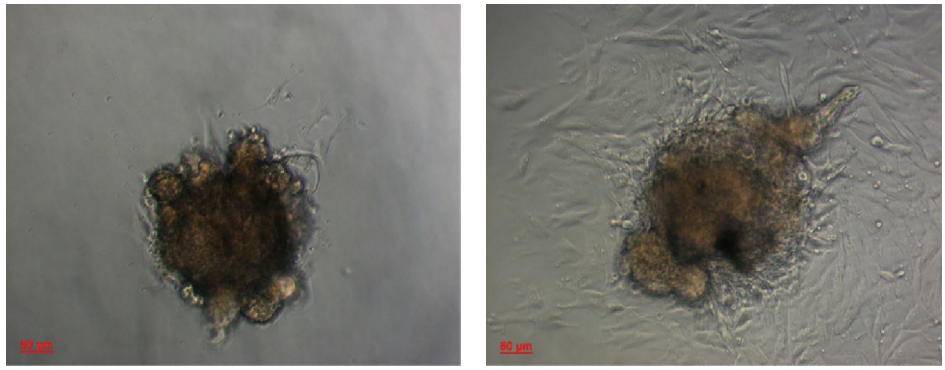
2.6. Treatment of INS-1 Cells with ISC-SN. Insulin-producing β-cell line (INS-1 832/13) cells were seeded into RPMI-1640 medium with L-glutamine containing 10% FBS. Upon reaching confluence, INS-1 cells were treated with or without 35% ISC-SN for up to 48 h. After exposure to ISC-SN, INS-1 cells were washed in RPMI 1640, and the following experiments were performed, representing $n = 3$ to 4.

2.6.1. 5-Ethynyl-2′-deoxyuridine (EdU) Incorporation Assay. EdU incorporation assay was performed as previously described [44]. In brief, EdU (Molecular Probes, Eugene, OR, USA) was added 4 h before the experiments ended and stained. Photos of eight different areas in each well were taken using a microscope with 200x magnification. The average percentage of $EdU^+/DAPI^+$ was calculated.

2.6.2. Propidium Iodide (PI) Staining. After removing the medium, INS-1 cells were incubated with PI (20 μg/mL) and Hoechst (2 μg/mL) (Sigma, St. Louis, MO, USA) for 10 min in the dark. Photos of eight different areas in each well were taken using a microscope with 200x magnification. The average PI staining rate was expressed as the percentage of PI-positive nuclei compared with the total cell count.

2.6.3. Caspase-3 Fluorometric Assay (CFA). CFA was performed according to the manufacturer's instructions (R&D, Minneapolis, MN, USA). CFA was performed in a 96-well flat-bottom microplate. After incubation with the substrate for 1.5 h, the plate was read on a fluorescent microplate reader equipped with filters set at 400 nm/505 nm (excitation/emission wavelengths). Data were normalized with total protein content in each well and expressed as OD/μg/μL.

2.6.4. Quantitative RT-PCR (qRT-PCR). Total RNA of INS-1 cells was extracted using TRIzol reagent (Invitrogen). The primer sequences used for amplification of genes encoding insulin and GAPDH are listed in Table 1. qRT-PCR analyses were performed using a standard SYBR-Green PCR kit protocol on a Step One Plus system (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The relative level of insulin transcripts was calculated and normalized to GAPDH, with at least four repeats per experimental group.



(a) (b)

Figure 1: (a) Some stellate shape-like cells began to grow out of four-month-old GK rat islets after 48 h of culture. (b) These cells exponentially proliferated and migrated out from the islets after 72 h, at 100x magnification ($n = 3$).

Table 1: Primer sequences of genes encoding insulin and GAPDH.

Gene	Primers
Insulin	5′-ATTGTTCCAACATGGCCCTGT-3′ 5′-TTGCAGTAGTTCTCCAGTT-3′
GAPDH	5′-TGTTCCTACCCCCAATGTGTCCGTC-3′ 5′-CTGGTCCTCAGTGTAGCCCAAGATG-3′

2.6.5. Potassium-Stimulated Insulin Secretion (KSIS) Assay. Measurements of potassium-stimulated insulin secretion (KSIS) by INS-1 cells were performed as described [13, 45]. Briefly, After preincubation in glucose-free Krebs-Ringer bicarbonate buffer for 1 h, cells were treated with low glucose (2.8 mmol/L) and high potassium (50 mmol/L) for 1 h. Insulin secretion after stimulation and insulin content in the cell lysate were measured using an insulin radioimmunoassay kit (Beijing Technology Company, Beijing, China). Data were normalized to the cellular insulin content and expressed as a percentage.

2.6.6. Measurement of Cytokines in ISC-SN. To determine the cytokines present in ISC-SN, the RayBio Biotin Label-Based Rat Antibody Array 1 (RayBiotech, Norcross, GA, USA) was performed according to the manufacturer's recommendations.

2.7. Statistical Analysis. Data are presented as the mean ± SE. Statistical significance was determined by unpaired Student's t-test or ANOVA, followed by the Bonferroni–Dunn post hoc test. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the Statistical Product and Services Solutions (SPSS) package (Version 11.5, SPSS Science, Chicago, IL, USA).

3. Results

3.1. Isolation and Characterization of ISCs. After 48 h of culture, cells with triangular shapes and large nuclei began to grow out of GK rat islets (Figure 1(a)). When the culture period was extended, these cells migrated away from the islet (Figure 1(b)). In addition, these stellate cells had a shorter doubling time (28 h). To determine these cells markers, immunofluorescence staining was performed. The ISC was positive for α-SMA (Figure 2(a)), vimentin (Figure 2(b)), and desmin (Figure 2(c)), thereby resembling the pattern of PSCs (Figures 2(d), 2(e), and 2(f)).

3.2. The DGE Profile of ISC Compared with PSC. Taking advantage of the DGE performed by the BGI Tech, we observed that the relative mRNA expression levels of ISCs were similar to PSCs mRNA expression pattern in intermediate filaments (*α-SMA*, *vimentin*, and *desmin*), ECM (*Procollagen, Pro-α-1 collagen type, Collagen α-1 type IX, Collagen α-1 type XI, Collagen α-1 type III, Fibronectin, Laminin, Tenascin, TIMP1, TIMP2, MMP1, MMP2, MMP14, RECK*, and *TUBA6*), cytokines (*IL-1β, IL-6, IL-7, IL-15, IL-18, TGF-α, TGF-β1, TGF-β2, TGF-β binding protein 2, TGF-β binding protein 3, HGF, PDGF-A, PDGF-B, CTGF, RANTES, MCP-1, ET-1*, and *VEGF*), signal transduction (*Smad1, Smad2, Smad7, Smad8, ERK1, ERK3*, and *ERK5*), integrins (*integrin α-1, integrin α-E2, integrin α-M, integrin α-v, integrin β, integrin β-3, integrin β-5*, and *integrin associated protein*), cytokine receptors (*TGFβR type1, PDGFRβ, PGFR1β, FGFR1, IL-3Rβ*, and *ActivinR*), and *PPARγ*. However, there were 1657 genes differentially expressed (600 genes upregulated and 1057 genes downregulated) of ISCs compared with that of PSCs, which enriched GO terms in metabolic pathways (196, 13.75%), pathways in cancer (63, 4.42%), MAPK signaling pathway (48, 3.37%), focal adhesion (46, 3.23%), regulation of actin cytoskeleton (44, 3.09%), biosynthesis of secondary metabolites (39, 2.74%), Alzheimer's disease (38, 2.67%), cell cycle (29, 2.04%), tight junction (37, 2.6%), vascular smooth muscle contraction (34, 2.39%), microbial metabolism in diverse environments (34, 2.39%), and chemokine signaling pathway (33, 2.32%), as well as Wnt signaling pathway (32, 2.5%) (for more information please see Supplementary Table 1 in the Supplementary Material available online at http://dx.doi.org/10.1155/2016/6924593).

3.3. ISC-SN Impaired INS-1 Cell Survival. Using EdU incorporation assay, the effects of ISC-SN on INS-1 cell proliferation were assessed. The proliferative capacity of INS-1 cells

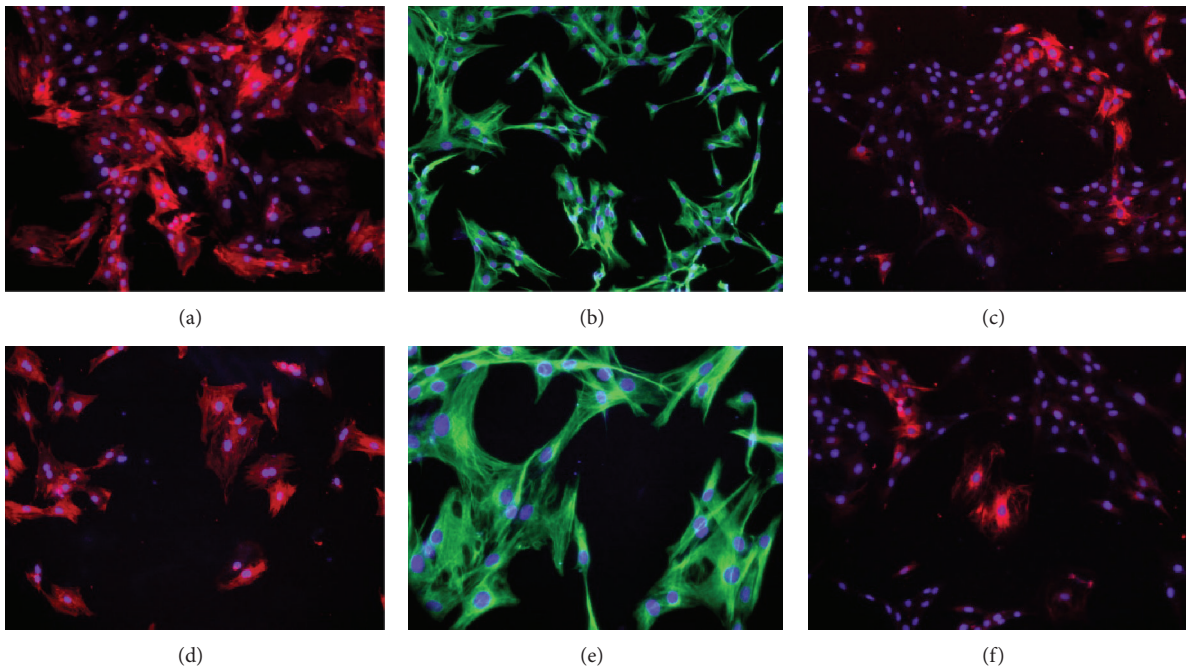


Figure 2: Stellate shape-like cell expression of (a) α-SMA, (b) vimentin, and (c) desmin and PSC expression of (d) α-SMA, (e) vimentin, and (f) desmin. Representative images for α-SMA and desmin staining were red, and those for vimentin were green at 100x magnification.

treated with ISC-SN (0.09 ± 0.01) was more significantly reduced than that in the control group (0.16 ± 0.02, $P < 0.01$) (Figures 3(a) and 3(b)).

To observe the effects of ISC-SN on INS-1 cell survival, apoptosis in INS-1 cells incubated with ISC-SN was detected by PI staining and CFA. As shown in Figure 4(a), a higher number of PI-positive INS-1 cells were observed in ISC-SN group. Quantitative analysis of PI-positive index (Figure 4(b)) showed that the dead cells in the ISC-SN group (0.18 ± 0.03) were 2.6-fold higher than those in the control (0.07 ± 0.02) groups. Caspase-3 activity in the ISC-SN groups (338.09 ± 62.51) was significantly higher than that in control groups (80.40 ± 16.59, $P < 0.01$) (Figure 4(c)).

3.4. ISC-SN Reduced Insulin Secretion of INS-1 Cells. To investigate the effects of ISC-SN on beta-cell function, insulin mRNA expression and secretion in INS-1 cells incubated with ISC-SN for 24 h were evaluated. Figure 5(a) showed that the insulin mRNA expression in INS-1 cells treated with ISC-SN (0.21 ± 0.10) was more significantly decreased than that in the control group ($P < 0.01$). In response to potassium challenge, insulin secretion in INS-1 cells incubated with ISC-SN (1.03% ± 0.23%) was more significantly reduced than that in control groups (1.71% ± 0.17% μIU/mL, $P < 0.01$) (Figure 5(b)).

3.5. Cytokine Profile of ISC-SN. To determine the cytokines that were highly expressed in ISCs-SN, RayBio Biotin Label-Based Rat Antibody Array 1 was performed. Cytokines, such as integrin $\alpha M\beta 2$, IFN-γ, MCP-1, MMP-2, PDGF, TNF-α, TLR4, and TROY, were highly expressed in both ISCs-SN and PSCs-SN (Table 2), whereas CD106, IL-1β, CXCR4, IL-3, IL-5, and MIF were highly expressed in ISCs-SN (Table 3).

Table 2: Cytokines were highly expressed in both ISC-SN and PSC-SN. ISCs-SN/PSC-SN was quantified by mean fluorescence intensity (MFI) using RayBio Biotin Label-Based Rat Antibody Array 1 assay. MFI ≥ 1.5-fold of negative control was considered as highly expressed.

	ISCs-SN (MFI)	PSCs-SN (MFI)
Negative control	5828.58	6319.81
Integrin alpha M beta 2	88599.0	75866.5
IFN-gamma	17506.0	16974.0
MCP-1	17223.0	24933.5
MMP-2	13303.0	22384.5
PDGF	20689.0	28034.5
TNF-alpha	17654.5	24232.0
TLR4	69218.5	48604.0
TROY	96330.0	104265.0

4. Discussion

In this study, we suggested an effective method for isolating ISCs from fibrotic islets of four-month-old GK rats. We observed a significant overlap and differentially expressed genes of ISCs and PSCs. In addition, ISCs exerted deleterious effects on beta-cells.

Previous studies have proven the existence of islet fibrosis in T2DM patients and rodent animal models, including GK

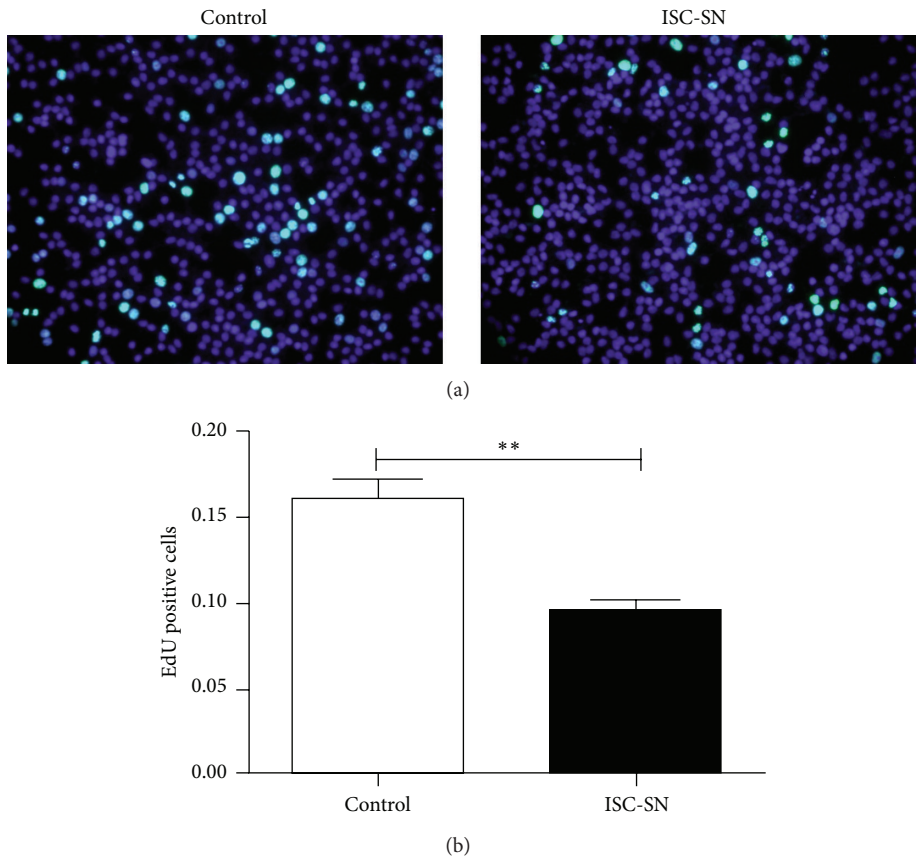


Figure 3: Effect of ISC-SN on INS-1 cell proliferation. (a) Cell proliferation was performed using EdU incorporation assay after treatment of INS-1 cells with ISC conditional medium for 48 h. Representative images for EdU staining (green) and nuclei labeled by DAPI (blue) at 200x magnification. (b) Data were expressed as mean ± SE ($n = 3$), $^{**}P < 0.01$.

Table 3: Cytokines were highly expressed in ISCs-SN than in PSCs-SN. MFI of ISCs-SN ≥ 1.5-fold of PSCs-SN was considered as highly expressed.

	ISCs-SN (MFI)	PSCs-SN (MFI)	Fold
Negative control	5828.5	6319.8	
CD106	47241.0	10725.0	4.40
IL-1β	22605.5	10431.0	2.24
CXCR4	23640.0	10119.5	2.34
ICAM-1/CD54	41550.0	12306.5	3.38
ICK	23586.0	11006.5	2.14
IL-3	12685.0	5296.0	2.40
IL-5	10394.0	6536.5	1.59
MIF	37598.0	17121.5	2.20

rats [3, 4, 8], a spontaneous T2DM model [46, 47]. *In vivo* studies showed that PSCs are present in rat islets and are critically important in the progression of islet fibrogenesis [10, 11, 24–27]. Evidence for the involvement of PSCs in islet fibrosis is mainly based on staining of α-SMA and/or GFAP for marker detection [25, 48, 49]. Islet fibrosis is possibly caused by other cell types that express the same markers, such as circulating fibrocytes. To determine which cell types are present in fibrotic islets of T2DM, islets from four-month-old GK rats were isolated and cultured. Although most islets in 16-week-old GK rats were significantly deformed with massive fibrosis, 90 ± 16 islets were harvested per pancreas using handpicking after mild digestion with collagenase V for 15 min to 18 min. As shown in Figure 1, stellate-like cells began to grow out of the islets after 48 h of culture, and these cells often exhibited a triangular shape and a large nucleus. Immunofluorescence staining demonstrated that these cells were positive for α-SMA, vimentin, and desmin, thereby resembling the protein expression profile of PSCs [15, 16]. Given that these cells were isolated from fibrotic islets of GK rats, which were presumably already influenced by the surrounding islet microenvironment in T2DM, were highly similar to activated PSCs. The isolation of ISCs from fibrotic islets in GK rats may provide a tool to investigate the interactions between ISCs and pancreatic beta-cell *in vitro*.

The interesting results in this study are the deleterious effects of ISC on INS-1 cell function and survival. Previous study has clearly shown that PSCs reduced insulin expression and induced apoptosis in pancreatic beta-cell [37], and high glucose aggravates the detrimental effects of PSCs on beta-cell function [13]. In this study, our data indicated

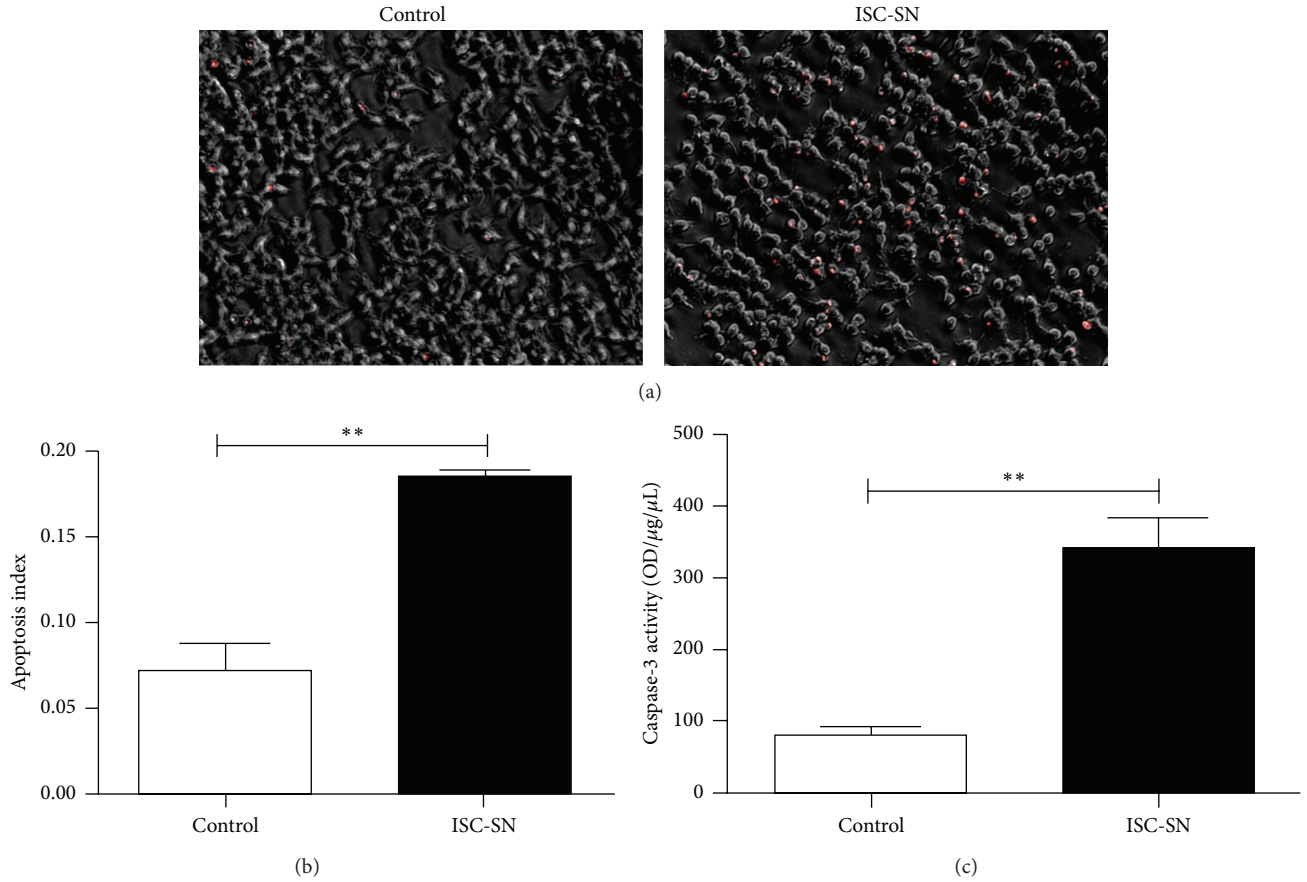


Figure 4: Effect of ISC-SN on INS-1 cell apoptosis. (a) Cell apoptosis was determined by PI staining (red), (b) PI-staining index, and (c) caspase-3 activity in INS-1 cells cultured with ISC-SN for 48 h. Data are expressed as mean ± SE ($n = 3$), $^{**}P < 0.01$.

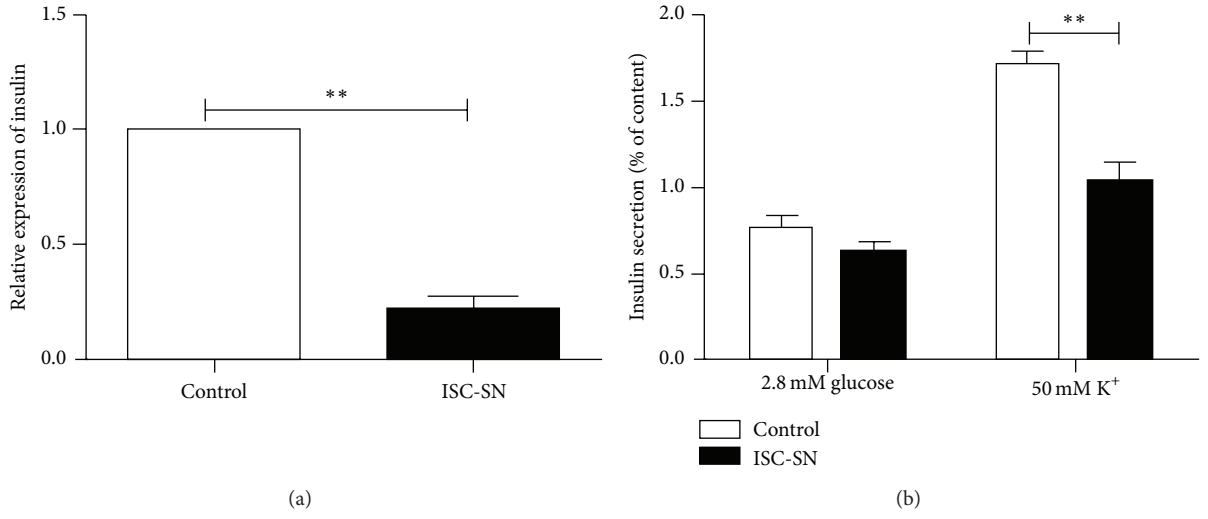


Figure 5: Effects of ISC-SN on insulin mRNA transcript and INS-1 cell function. (a) qRT-PCR analysis of insulin mRNA expression and (b) KSIS assay on INS-1 cells cultured with ISC-SN for 24 h. Insulin mRNA data were normalized by GAPDH gene, and insulin secretion data were normalized by cellular insulin content. Data are expressed as mean ± SE ($n = 4$ to 5), $^{**}P < 0.01$.

that ISC also exhibited deleterious effects on INS-1 beta-cells by reducing insulin mRNA transcription and inhibiting insulin secretion. In addition, ISCs significantly decreased cell proliferation and increased cell apoptosis. To determine the factors that possibly contribute to the effect of ISCs on INS-1 cells, cytokine profiles in ISCs were assessed. ISCs-SN was shown to contain high levels of cytokines, such as IFN-γ and TNF-α, which are well-known factors mediating or promoting cell death [50–55]. Our data also show that ISCs-SN contained higher levels of cytokines than PSCs-SN, such as IL-1β, which can impair beta-cell function and induce cell death at higher concentrations [56].

5. Conclusion

In conclusion, our *in vitro* study shows that fibrotic islets of GK rats contain a population of stellate cells resembling but not identical to PSCs, which exerted deleterious effects on beta-cells by inducing beta-cell death and suppressing insulin production and cell proliferation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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Clinical Study

The Initial Assessment of Daily Insulin Dose in Chinese Newly Diagnosed Type 2 Diabetes

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Background. It has been well accepted that insulin therapy is the ideal treatment for newly diagnosed diabetic patients. However, there was no study about assessment of the initial insulin dosage in new onset Chinese patients with type 2 diabetes. *Research Design and Methods*. 65 newly diagnosed patients with type 2 diabetes (39 males/26 females; HbA1c $\geq 11.80 \pm 0.22\%$) were investigated. All patients had random hyperglycaemia (at 21.8 ± 3.9 mmol/L) on the first day of admission and received insulin infusion intravenously (5 U/per hour). When the blood glucose level dropped to around 10 mmol/L, patients were then transferred to continuous subcutaneous insulin infusion (CSII). The reduction of blood glucose levels in response to per unit of insulin (RBG/RI) was recorded. The target glucose level was achieved in about 3 days. The total daily insulin dose (TDD) and basal insulin dose (TBD) were calculated. *Results*. TDD was 45.97 ± 1.28 units and TBD was 19.00 ± 0.54 units. TBD was about 40% of the total daily insulin requirement. There was a negative correlation between the ratio of RBG/RI and TDD. *Conclusions*. TDD was correlated with blood glucose reduction in response to intravenous insulin infusion in Chinese new onset patients with type 2 diabetes.

1. Introduction

It has been reported that the prevalence of diabetes in Chinese adults was up to 11.6% by the China Noncommunicable Disease Surveillance Group in 2013 [1]. Patients with type 2 diabetes in Asian country mainly have impaired β-cell function rather than insulin resistance compared to those in western country [2]. Therefore, early intensive insulin therapy is used as the ideal therapy to restore the β-cell function and to eliminate glucotoxicity in patients with newly diagnosed type 2 diabetes [3, 4].

Intensive insulin therapy normally includes continuous subcutaneous insulin infusion (CSII) and multi-daily insulin injections therapy (MDI). Patients with CSII present more prominent reduction in HbA1c and glycaemia with less hypoglycaemic events compared to MDI [5]. CSII is widely used in patients with type 1 diabetes, pregnant women with gestational diabetes mellitus, and patients with type 2 diabetes in preoperation or those with poor glycaemic control [6]. Optimal insulin pump therapy is determined by accurate setting of total daily insulin dose (TDD), basal and bolus insulin dose [7]. It is primary to get the TDD which is normally estimated by body weight (0.4–0.5 IU/kg per day) according to the Chinese guideline of insulin pump in 2010 [8]. Basal insulin dose (TBD) is calculated by the certain ratio of TDD. It has been shown in a Japanese study that TBD is about 30% of TDD in patients with type 1 diabetes who use the insulin pump [9]. The bolus dosage is adjusted according to carbohydrate factor (CarbF) with "450 rule" and glucose correction factor (CorrF) with "1500 rule." However, there was no study about the initial insulin dose in newly diagnosed patients with type 2 diabetes.

The purpose of this study was to assess total daily insulin requirement and the impact factors in newly diagnosed patients with type 2 diabetes, who were on transient CSII therapy in China.

2. Methods

2.1. Subjects (Table 1). All participants with type 2 diabetes were diagnosed by World Health Organization criteria. None

Table 1: Clinical characteristics of the subjects.

Variables	Value
M/F (n)	39/26
Age (years)	49.7 ± 2.1
Weight (kg)	66.52 ± 1.56
Height (cm)	165.6 ± 1.08
BMI (kg/m^2)	24.15 ± 0.43
WHR	0.96 (0.89–0.96)
HbA1c (%)	11.8 ± 0.22
FCP (ng/mL)	1.53 (0.96–2.52)
Cr (μmol/L)	58.49 (41.80–68.95)
BUN (mmol/L)	6.54 ± 0.72
UA (μmol/L)	296.4 ± 14.65
TC (mmol/L)	5.48 ± 0.22
TG (mmol/L)	2.08 (1.09–2.69)
LDL (mmol/L)	3.54 ± 0.20

Data represent means ± SE, or median (interquartile range 25–75%).

had eating disorders, diabetic ketoacidosis, hyperosmolar status, and other acute diabetic complications. All patients have the negative GAD antibody levels.The anion gap in the initial electrolytes was 12.2 ± 1.6. (The normal range is 8–16.) They have the characteristics as follows: 39 males/26 females; age 49.72 ± 2.1 years old, body weight 66.5 ± 1.6 kg, height 165.6 ± 1.1 cm, BMI 24.2 ± 0.4 kg/m^2, and glycated haemoglobin (HbA1c) 11.80 ± 0.2%.

2.2. Protocol. A total of 65 Chinese newly diagnosed patients with type 2 diabetes were included in this study. All the patients were hospitalized in Department of Endocrinology and Metabolism of Shanghai Renji Hospital between July 2011 and December 2013. All the patients had random postprandial glucose levels at 21.8 ± 3.9 mmol/L on the first day of admission. They were given intravenous insulin infusion at the initial rate of 5 U/h (25 U insulin/250 mL saline). When patients received intravenous insulin infusion, none of antidiabetic mediations were used. We monitored the capillary blood glucose every 30 minutes. The intravenous insulin infusion was stopped when the capillary glucose levels were dropped to around 10 mmol/L. The reduction of blood glucose and the dose of insulin were recorded and the ratio of the reduction of blood glucose/per unit of insulin (RBG/RI) was calculated. All patients were switched to insulin pump (Paradigm 712 pump, Medtronic, Northridge, CA) with rapid-acting insulin (Insulin Aspart Injection, Novo Nordisk) for 72 hours. Capillary blood glucose levels were tested eight times daily (before meal, after meal, bedtime, and 2 a.m.). In 72 hours, the basal insulin rate and the bolus insulin dose were adjusted according to the capillary blood glucose levels. The target fasting glucose and 2 h postprandial glucose were set at 7 mmol/L and 10 mmol/L. At the end of 72 hours, all the patients got the target glycaemia. The insulin pump was stopped. TBD and bolus insulin dose for all of them were recorded. During the hospitalization, the meals were prepared by dietitians (total energy intake 20–25 kcal/kg, 50–60% from carbohydrate, 15–20% from protein, and 20–25% from fat). No additional food was consumed unless hypoglycaemic events occurred.

2.3. Statistical Analysis. The data were shown as means ± standard error. The mainly statistical methods included bivariate correlation analysis and multiple regression analysis with SPSS 20.0. P values < 0.05 were considered significant.

3. Results

3.1. Insulin Dose. When the target glucose level was achieved, the TDD was 45.97 ± 1.28 U and TBD was 19.00 ± 0.54 U. The percentage of TBD was 41.74 ± 0.87%. Total bolus dose was 27.01 ± 0.96 U (prebreakfast bolus 11.87 ± 0.44 U, prelunch bolus 6.79 ± 0.29 U, and predinner bolus 8.35 ± 0.36 U). The percentage of prebreakfast, prelunch, and predinner bolus to total bolus dose was 44.15 ± 0.71%, 25.19 ± 0.58%, and 30.65 ± 0.65%, respectively.

3.2. Correlation between the Ratio of RBG/RI and Insulin Dose. There was a negative correlation between the ratio of RBG/RI and TBD ($r = -0.710$, $P < 0.01$) and TDD ($r = -0.546$, $P < 0.01$). However, there was no relationship between the ratio of RBG/RI and total bolus dose, but the ratio of RBG/RI was negatively related to prebreakfast (−0.320, $P < 0.01$) and prelunch bolus (−0.292, $P < 0.05$). It tended to be related to predinner bolus ($r = -0.211$). TBD presented logarithm relevant to the ratio of RBG/RII ($r = -0.740$, $P < 0.01$) (Figure 1). Body weight, BMI, fasting blood glucose, and TG were related to the TDD and TBD. However, HbA1c was not the independent factors for TDD or TBD.

3.3. Stepwise Multiple Regression Analysis. TBD was independently correlated with lg (the ratio of RBG/RII) ($\beta = -0.675$, $P < 0.01$) and age ($\beta = -0.377$, $P < 0.05$) with stepwise multiple regression analysis. Regression equation was y(TBD) = 25.62 − 15.927 ∗ lg[x1 (the ratio of RBG/RII)] − 0.101 ∗ x2 (age).

4. Discussion

Our study is the first to show that the initial dose of insulin is related to the ratio of RBG/RI in Chinese new onset patients with diabetes who use the insulin pump therapy. The basal insulin dose is about 40% of total daily insulin requirement.

In newly diagnosed Chinese patients with type 2 diabetes, insulin secretion, in particular at early phase, is normally reduced due to the impaired β-cell function [10]. Hyperglycaemia caused glucotoxicity which leaded to further decline of β-cell function [11]. Patients with type 2 diabetes enrolled in our study were drug-naïve with random hyperglycaemia (blood glucose levels 21.8 ± 3.9 mmol/L and HbA1c 11.8 ± 0.2% as shown in Table 1). BMI was in the range of 23.8 to 24.2 kg/m^2 (as shown in Table 1). According to the Chinese Adult Obesity Guide, overweight is defined as BMI over 24 kg/m^2. Therefore, patients in our study were normal body

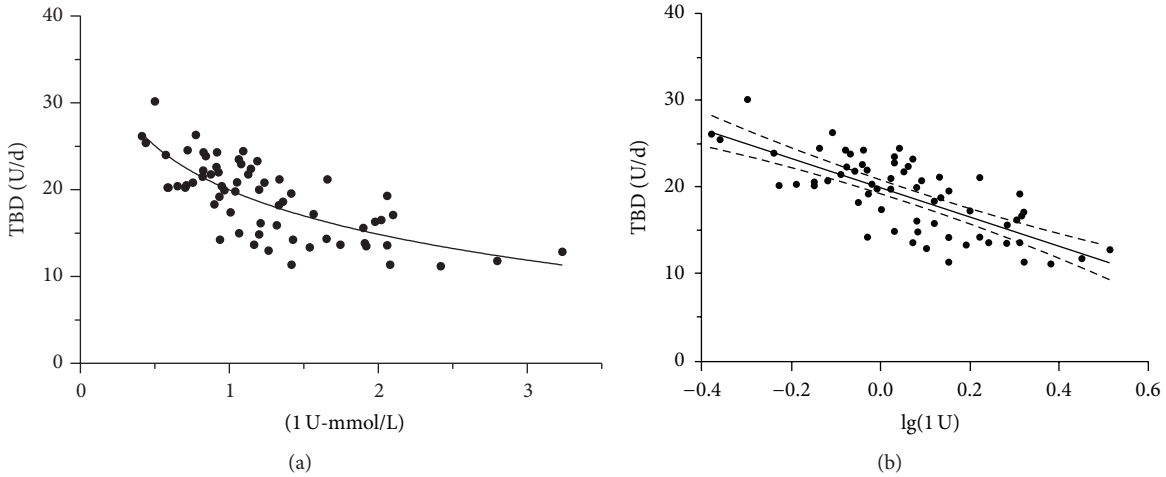

Figure 1: (a) TBD was decreased with the ratio of RBG/RII: $r = -0.710$ ($n = 65$), $P < 0.01$. (b) Correlations of the ratio of RBG/RII (log transformed) with TBD in 65 subjects: $r = 0.740$, $P < 0.01$.

weight or slightly overweight. It appears primary for those diabetic patients to obtain the target glucose level and reserve β-cell function with insulin therapy.

Intravenous infusion of insulin was previously used to achieve glycaemic target for patients in the Intensive Care Unit [12] and those with diabetic ketoacidosis due to the rapid action and shorter half-life [13]. In order to lower blood glucose level in the shorter period, we infused insulin intravenously before insulin pump therapy. Although the decline rate of glucose levels may be related to many factors such as the relative degree of insulin resistance, the differences in the clearance of glucose in the urine, and the amount of fluid infused or ingested into the patient, the RBG/RI ratio has the strongest correlation with TBD among all those factors (Figure 1). We aimed to find a practical method in calculation of the initial dose of insulin in the clinical work. Blood glucose levels at admission were fairly high. Insulin sensitive factor itself would be variable due to glucotoxicity. It was not reasonable to explore the relationship of β-cell function with TDD during the 72 hours of CSII. It is not surprising that body weight was correlated with TDD. We estimated the insulin dose according to the body weight (0.4–0.5 IU/kg per day) in previous calculation. We also found that TBD was independently correlated with lg (the ratio of RBG/RII) and age with stepwise multiple regression analysis (as shown in Figure 1). Therefore, we did regression equation as y(TBD = 25.62 − 15.927 ∗ lg(the ratio of RBG/RI) − 0.101 ∗ age) by the software. It may provide the direction that we could easily calculate the insulin dose in the future clinical work.

Chinese has changed eating habit from high-carbohydrate to high protein/fat dietary pattern in recent years. However, Chinese populations still have rice and noodle as major energy resource, particularly in breakfast. It has been reported that newly diagnosed Chinese patients with type 2 diabetes consumed higher proportion of carbohydrates (over 65%) in the diet compared to that recommended by Chinese and international dietary guidelines for macronutrients [14]. It is not surprising that Chinese new onset diabetic patients have higher occurrence of the isolated postprandial hyperglycaemia than fasting hyperglycaemia (44.1% of the men and 50.2% of the women) [15]. Accordingly, the percentage of bolus insulin dose was higher in Chinese population than that in western country.

This study has some limitations. Firstly, we did not use a continuous glucose monitoring system (CGMS) before and after the treatment in our study. However, it has been shown that eight-point glucose test daily is comparable to CGMS [16]. Secondly, we did not clarify the correlation of energy intake with premeal bolus of insulin. The dietitian prepared the meal according to the body weight. We already found that there was a weak correlation between body weight and insulin dose. Finally, the duration of this study was relatively short. Longer term studies and larger studies are required in the future research.

In conclusion, basal insulin requirement is 40% TDD in newly diagnosed patients with type 2 diabetes in China. TDD was related to the rate of blood glucose reduction in response to initial intravenous insulin infusion.

Conflict of Interests

There is no conflict of interests to disclose.

Authors' Contribution

Jing Ma and Huan Zhou contributed equally to this work.

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Research Article

Circulating Levels of Betatrophin and Irisin Are Not Associated with Pancreatic β-Cell Function in Previously Diagnosed Type 2 Diabetes Mellitus Patients

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Betatrophin and irisin are two recently identified hormones which may participate in regulating pancreatic β-cell function. However, the associations of these two hormones with β-cell function remain unclear. The present study aims to demonstrate the associations of circulating betatrophin and irisin levels with β-cell function, assessed by the area under the curve (AUC) of C-peptide, and the possible correlation between these two hormones in previously diagnosed type 2 diabetes mellitus (T2DM) patients. In total, 20 age-, sex-, and body mass index- (BMI-) matched normal glucose tolerance (NGT) subjects and 120 previously diagnosed T2DM patients were included in this study. Partial correlation analysis was used to evaluate the relationships between these two hormones and indexes of β-cell function and insulin resistance. Our results showed that betatrophin levels were significantly elevated, while irisin levels were significantly decreased, in patients with T2DM compared with NGT subjects. However, partial correlation analysis showed that betatrophin levels did not correlate with β-cell function-related variables or insulin resistance-related variables before or after controlling multiple covariates, while irisin correlated positively with insulin sensitivity but is not associated with β-cell function-related variables. Besides, no correlation was observed between betatrophin and irisin levels. Hence we concluded that betatrophin and irisin were not associated with β-cell function in previously diagnosed T2DM patients.

1. Introduction

It has been suggested that the best treatment, and a potential cure, for both type 1 mellitus (T1DM) and type 2 diabetes mellitus (T2DM), is to replace or regenerate the pancreatic β-cell mass [1]. Interestingly, two recently identified hormones, betatrophin and irisin, might be involved in this process [2], although their specific physiological effects on pancreatic β-cell have not been confirmed. Betatrophin (also known as ANGPTL8, Lipasin, RIFL, EG624219, and TD26) [3, 4] was found to be a novel stimulator of β-cell by Yi et al. in a S961-induced insulin resistance mouse model [5]. Overexpression of betatrophin in mice livers was reported to induce a striking increase of β-cell proliferation rate 17-fold higher than the controls. However these inspiring discoveries were later challenged by either genetic ablation of betatrophin or its overexpression, which showed no significant effect on β-cell mass in mice [6, 7]. Despite of the facts above, studies of betatrophin in human subjects were also limited and nonconsistent, and researches focusing on correlation between betatrophin and β-cell function in T2DM human subjects were lacking. Therefore, the relevance of betatrophin with β-cell function needs to be further warranted, especially in human subjects.

Irisin was initially described as a protective factor against diet-induced weight gain by browning of white adipose tissue (WAT) [8]. Interestingly, a moderate increase in circulating irisin levels could also improve diet-induced insulin resistance [8], which indicated the potential important role of irisin in regulating glucose homeostasis. However, only few studies explore the association of irisin with β-cell function, although there have been numerous studies conducted in

diabetes subjects. A recent study reported that serum irisin levels were closely related to homeostasis model assessment-β (HOMA-β) in normal glucose tolerance (NGT) subjects, suggesting that irisin may play a crucial role in β-cell function. However, similar relationship was not observed in diabetic subjects [9]. Considering that HOMA-β could not reflect β-cell function accurately, the relationship between irisin and β-cell function, assessed by a more accurate method, in diabetes subjects remains unclear and needs further investigation.

Furthermore, Zhang et al. discovered that irisin could promote the expression of betatrophin, thus raising the possibility that the euglycemic effect of irisin was partially mediated by the upregulation of betatrophin through β-cell proliferation [10]. However, the relationship between betatrophin and irisin in human subjects has not been clarified. Therefore, the present study aims to demonstrate the association of circulating betatrophin and irisin levels with pancreatic β-cell function, assessed by the gold standard measure of β-cell function, the area under the curve (AUC) of C-peptide [11], and the possible correlation between betatrophin and irisin levels in previously diagnosed T2DM patients.

2. Methods

2.1. Subjects. This cross-sectional study randomly recruited 120 previously diagnosed T2DM patients with durations ≥ 1 year at Qilu Hospital of Shandong University from May 2014 to November 2014 after a review of their medical records based on the following 1999 WHO criteria: fasting blood glucose (FBG) ≥ 126 mg/dL (7.0 mmol/L) and/or 2 h postprandial blood glucose ≥ 200 mg/dL (11.1 mmol/L) [12]. The following exclusion criteria were applied: patients with (1) T1DM, secondary diabetes, or specific types of diabetes or diabetic ketoacidosis, lactic acidosis, or a hyperglycemic hyperosmolar state; (2) Diabetic foot or inflammatory or infectious diseases; (3) acute cerebral infarction or acute myocardial infarction; (4) familial hypercholesterolemia, and samples with visible lipidemia and hemolysis; and (5) heart failure and severely impaired liver or renal function. Healthy age-, sex-, and body mass index- (BMI-) matched normal glucose tolerance (NGT) controls ($n = 20$), without a family history of T2DM, were recruited by advertising. Written informed consent was obtained from all subjects, and the study was approved by the ethics committee of the Qilu Hospital of Shandong University.

2.2. Clinical Data Collection. The computerized patient record system of Qilu Hospital was used to collect data regarding the demographic characteristics and previous medical histories of subjects. Antidiabetic medications were included in the following categories: insulin, insulin secretagogues, and others (thiazolidinedione (TZD), metformin, and alpha glucosidase inhibitor). BMI was determined by dividing the weight by the height squared (kg/m^2). Blood pressure (BP) was measured 3 consecutive times (OMRON Model HEM-752 FUZZY, Omron Company, Dalian, China) using the left arm after the subject had remained seated for at least 5 min, and the average reading was used for the analysis. Fasting blood samples were collected after a 10-hour fast and before the ingestion of breakfast and medication. FBG, total cholesterol (TC), triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels were measured by an automatic analyzer (TOSHIBA TBA-40F, Toshiba, Japan). HbA1C was measured by high-performance liquid chromatography (BIO–RAD VARIANT II, Bio-Rad, USA). Plasma betatrophin levels were determined by an ELISA (Wuhan Eiaab Science, Wuhan, China; Catalogue number E11644h). Plasma irisin levels were measured by ELISA (Phoenix Pharmaceuticals, Inc., Burlingame, USA; Catalogue number EK-067-29).

2.3. Islet β-Cell Function and Insulin Resistance Assessment. Any insulin treatment was stopped 10 hours before collecting fasting blood samples. 75-g oral glucose tolerance test (OGTT) was carried out for all T2DM patients and blood glucose and C-peptide were measured at 0, 30, 60, and 120 min after glucose administration. Plasma C-peptide levels were detected by a chemiluminescence immunoassay analyzer (Bayer ADVIA Centaur, Bayer, Germany). β-cell function was presented by AUC (including $AUC_{0-0.5}$, AUC_{0-1}, and AUC_{0-2}) of C-peptide release test calculated using the trapezoidal rule. Fasting C-peptide and glucose was used to calculate homoeostasis model assessment to estimate basal β-cell function (HOMA2-%B), insulin sensitivity (HOMA2-%S), and insulin resistance (HOMA2-IR) by the computerized HOMA2 model downloaded from http://www.ocdem.ox.ac.uk/ [13].

2.4. Statistical Analysis. The continuous variables with normal distribution are expressed as the mean ± standard error of mean (SEM), and the variables with nonnormal distribution are presented as the median (interquartile range). The categorical variables are presented as numbers (%). Normal distribution of the data was tested using the Kolmogorov-Smirnov test. Between-group differences were detected using one-way ANOVA (LSD) test (the continuous variables in normal distribution), Mann Whitney U-test (the skewed continuous variables), or chi-squared test (categorical variables). The correlations between variables were assessed using a Pearson correlation analysis by controlling for the covariates. $P < 0.05$ was considered statistically significant. All the above statistical analyses were performed with SPSS 16.0 software (SPSS Inc., Chicago, USA).

3. Results

3.1. Comparison of Betatrophin and Irisin Levels in NGT and T2DM Subjects. The glucose-related variables and levels of betatrophin and irisin in NGT and T2DM subjects were shown in Table 1. As expected, T2DM subjects showed higher FBG and HbA1c levels, compared with age-, sex- and BMI-matched NGT group. However, the levels of fasting insulin of T2DM subjects were also higher, although the fasting C-peptide levels were not significantly different between two groups. Notably, circulating betatrophin levels were significantly elevated (592.1 ± 37.5 versus 291.1 ± 37.3 pg/mL),

Table 1: Characteristics of NGT and T2DM subjects.

Characteristics	NGT (n = 20)	T2DM (n = 120)	P-value
Female [n (%)]	10 (45.5%)	52 (43.3%)	0.750
Age (years)	54.4 ± 2.9	56.3 ± 1.2	0.232
BMI (kg/m^2)	26.0 ± 0.5	26.3 ± 0.4	0.340
Systolic BP (mmHg)	128.3 ± 4.0	132.8 ± 1.5	0.186
Diastolic BP (mmHg)	72.1 ± 2.6	76.7 ± 1.1	0.109
FBG (mmol/L)	5.3 ± 0.1	7.8 ± 0.3	<**0.001**
HbA1c (%)	5.3 ± 0.1	8.6 ± 0.2	<**0.001**
Fasting insulin (mIU/L)	4.90 (3.2–7.6)	12.0 (7.5–17.8)	<**0.001**
Fasting C-peptide (ng/mL)	1.5 ± 0.1	1.6 ± 0.1	0.424
Betatrophin (pg/mL)	291.1 ± 37.3	592.1 ± 37.5	<**0.001**
Irisin (ng/mL)	4.7 ± 0.1	3.4 ± 0.1	<**0.001**

The data are expressed as the means ± SEM or median (interquartile range) or numbers (%). NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus; BMI, body mass index; BP, blood pressure; FBG, fasting blood glucose.

while irisin levels were significantly decreased (3.4±0.1 versus 4.7±0.1 ng/mL), in patients with T2DM compared with NGT subjects.

3.2. Comparison of Betatrophin and Irisin Levels in T2DM Subjects with Different β-Cell Function. The clinical characteristics and levels of betatrophin and irisin in T2DM subjects with different β-cell function were shown in Table 2. β-cell function was measured by the gold standard measure, the AUC of C-peptide [11], and we grouped the subjects to four quartiles according to the AUC of C-peptide levels [14]. In general, levels of BMI, fasting C-peptide, HOMA2-%B, HOMA2-IR, and TG were found to be positively associated with AUC_{0-2} values, and HbA1c, HOMA2-%S, the duration of diabetes, the percentage of insulin usage, and HDL-C levels were inversely associated. No significant differences among the groups in their BP, FBG, TC, and LDL-C were found. Notably, circulating levels of betatrophin and irisin were not significantly different among these four groups.

3.3. Correlations between Betatrophin Levels and Indexes of β-Cell Function and Insulin Resistance. The correlations between betatrophin levels and glucose-related variables based on the values of 120 individual T2DM subjects were shown in Table 3 and Figure 1. We observed that betatrophin levels did not correlate with β-cell function-related variables ($AUC_{0-0.5}$, AUC_{0-1}, AUC_{0-2}, and HOMA2-%B) or insulin resistance-related variables (HOMA2-%S and HOMA2-IR) in T2DM subjects before or after controlling multiple covariates.

3.4. Correlations between Irisin Levels and Indexes of β-Cell Function and Insulin Resistance. The correlations between irisin levels and glucose-related variables based on the values of 120 individual T2DM subjects were shown in Table 4 and Figure 2. Similar to the betatrophin, irisin levels were not associated with β-cell function either. However, it is positively associated with HOMA2-%S, even after controlling multiple covariates.

3.5. Correlations between Betatrophin and Irisin Levels in T2DM Subjects. Finally, as shown in Figure 3, we analysed the correlation between betatrophin and irisin levels in T2DM subjects. Unfortunately, no significant relationship was observed between these two hormones.

4. Discussion

For lacking direct evidence of the role of betatrophin in islet β-cell replication in the human model and the fact that there were relatively few clinical studies focusing on the above issue, we first measured the circulating betatrophin levels in age-, sex- and BMI-matched healthy NGT and T2DM subjects. Similar to what has been previously reported [15], betatrophin levels were almost doubled in patients with T2DM compared with NGT (592.1 ± 37.5 versus 291.1 ± 37.3 pg/mL). It is noteworthy that the levels of betatrophin reported by Espes et al. [16] and Chen et al. [17] were higher than those of ours despite the similar trends between T2DM and control subjects. These discrepancies might be due to the different age of subjects. As what was suggested by our study and the results of other studies [15, 16, 18], age seemed to be positively related to the levels of betatrophin. Age of the diabetic subjects in the studies of Chen et al. (60.7 ± 0.9 years) and Espes et al. (61.9 ± 1.7 years) is older than those of ours (56.3 ± 1.2 years) and thus might partially explain the difference of the results.

Moreover, we found there was no significant correlation between betatrophin and indices of β-cell function ($AUC_{0-0.5}$, AUC_{0-1}, AUC_{0-2}, and HOMA2-%B), Futhermore, no relationships between betatrophin and glycemic control indices such as FBG and HbA1c were found. Suggesting that betatrophin might not play an important role in regulating glucose homeostasis, which had been proved by mice models from Wang and colleagues [6]. They reported that mice knocked out for ANGPTL8/betatrophin showed no alterations in glucose homeostasis when fed either chow or high fat diet. Then more recently, Gusarova and colleagues further confirmed this issue by overexpressing betatrophin in mice livers and observed no significant alteration in β-cell expansion nor glucose metabolism [7]. Nevertheless, these results were obtained from diet or S961-induced insulin resistant mice models; the role of betatrophin on β-cell expansion under more extreme conditions of β-cell destruction is still under a veil, such as in diet or gene-deficient T2DM models. Furthermore, the results in mice cannot be fully applied to humans. Jiao et al. observed that betatrophin of mice failed to induce human β-cell replication, which raised a possibility that mouse and human betatrophin might undergo different posttranslational processing [19, 20]. Additionally, Chen et al. [17] claimed that circulating betatrophin levels were associated with markers of insulin

Table 2: Characteristics of the study participants by AUC of C-peptide quartiles in T2DM subjects.

Characteristics	Quartile 1 ($n = 30$)	Quartile 2 ($n = 30$)	Quartile 3 ($n = 30$)	Quartile 4 ($n = 30$)	P-value[a]
Female [n (%)]	18 (60.0%)	12 (40.0%)	9 (30%)	13 (43.3%)	0.127
Age (years)	61.6 ± 2.0	54.0 ± 2.6[b]	53.8 ± 2.4[b]	55.8 ± 3.0[b]	0.098
BMI (kg/m^2)	24.1 ± 0.8	25.6 ± 0.6	27.3 ± 0.8[b]	28.2 ± 0.9[bc]	**0.001**
Systolic BP (mmHg)	137.3 ± 3.3	129.1 ± 2.5	133.6 ± 3.7	131.4 ± 3.0	0.315
Diastolic BP (mmHg)	74.4 ± 2.3	74.5 ± 1.9	79.4 ± 2.9	78.6 ± 2.4	0.302
FBG (mmol/L)	7.8 ± 0.5	8.1 ± 0.5	7.7 ± 0.6	7.6 ± 0.4	0.527
HbA1c (%)	9.2 ± 0.4	8.9 ± 0.4	8.3 ± 0.3	8.1 ± 0.4[d]	**0.025**
Fasting insulin (mIU/L)	10.0 (5.1–15.4)	11.2 (6.3–16.8)	12.3 (8.6–16.5)	15.8 (11.1–20.1)	0.051
Fasting C-peptide (ng/mL)	0.6 ± 0.1	1.3 ± 0.1[b]	1.7 ± 0.1[bc]	2.6 ± 0.1[bcd]	<**0.001**
HOMA2-%B	21.7 (14.5–27.3)	39.7 (25.4–60.2)[b]	37.9 (29.1–63.0)[b]	82.9 (46.5–102.1)[bcd]	< **0.001**
HOMA2-%S	166.7 (130.2–274.8)	96.7 (71.0–131.7)[b]	72.5 (51.9–96.2)[bc]	44.2 (38.6–59.0)[bcd]	<**0.001**
HOMA2-IR	0.6 (0.3–0.7)	1.0 (0.8–1.4)[b]	1.4 (1.1–1.9)[bc]	2.3 (1.7–2.6)[bcd]	<**0.001**
Duration of diabetes (years)	13.2 ± 1.6	7.0 ± 1.1[b]	7.8 ± 1.4[b]	6.0 ± 1.0[b]	**0.001**
Insulin secretagogues treatment [n (%)]	11 (36.7%)	17 (56.7%)	20 (66.7%)	17 (56.7%)	0.110
Other antidiabetic medications [n (%)]	23 (76.7%)	21 (70.0%)	28 (93.3%)	26 (86.7%)	0.096
Insulin treatment [n (%)]	23 (76.7%)	16 (53.3%)	9 (30.0%)[b]	5 (16.7%)[bc]	<**0.001**
Triglyceride (mmol/L)	1.1 (0.7–1.5)	1.6 (1.1–2.3)[b]	1.7 (1.0–2.1)[b]	1.9 (1.4–2.6)[b]	<**0.001**
Cholesterol (mmol/L)	4.9 ± 0.2	4.6 ± 0.2	4.5 ± 0.2	5.0 ± 0.2	0.191
HDL-C (mmol/L)	1.5 ± 0.1	1.2 ± 0.1[b]	1.3 ± 0.1[b]	1.2 ± 0.0[b]	**0.001**
LDL-C (mmol/L)	2.8 ± 0.2	2.7 ± 0.1	2.6 ± 0.1	3.0 ± 0.1	0.217
Betatrophin (pg/mL)	637.0 ± 92.3	522.2 ± 70.1	633.9 ± 88.2	575.6 ± 70.4	0.720
Irisin (ng/mL)	3.6 ± 0.2	3.1 ± 0.2	3.1 ± 0.2	3.6 ± 0.2	0.073

The data are expressed as the means ± SEM or median (interquartile range) or numbers (%). AUC, area under the curve; BMI, body mass index; BP, blood pressure; FBG, fasting blood glucose; HOMA2-%B, homoeostasis model assessment of β-cell function; HOMA2-%S, homoeostasis model assessment of insulin sensitivity; HOMA2-IR, homoeostasis model assessment of insulin resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. [a]Difference between four groups; [b]$P < 0.05$ compared with Quartile 1 group; [c]$P < 0.05$ compared with Quartile 2 group; [d]$P < 0.05$ compared with Quartile 3 group.

Table 3: Partial correlations between betatrophin levels and glucose-related variables in T2DM subjects.

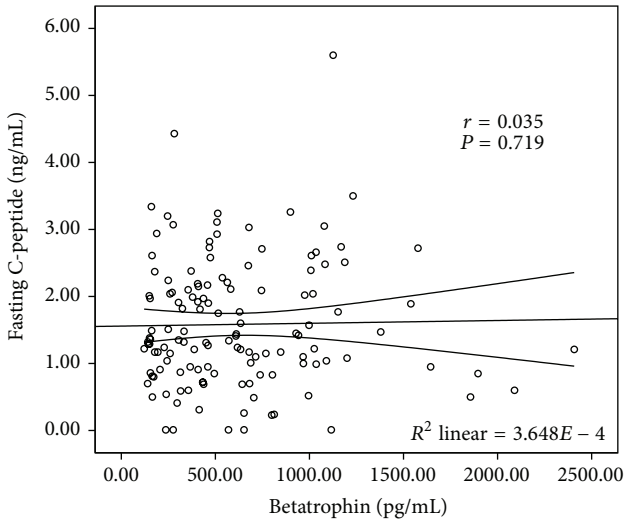
Characteristics	Model 1		Model 2		Model 3	
	r	P-value	Partial r	P-value	Partial r	P-value
HbA1c	0.026	0.765	0.129	0.174	0.057	0.566
FBG	−0.112	0.194	−0.053	0.577	−0.044	0.659
Fasting insulin	−0.080	0.354	−0.044	0.644	−0.103	0.296
Fasting C-peptide	0.019	0.828	0.015	0.878	0.035	0.719
$AUC_{0.5}$	0.007	0.936	0.032	0.738	0.058	0.555
AUC_1	0.005	0.961	0.023	0.809	0.056	0.572
AUC_2	−0.007	0.940	−0.002	0.984	0.035	0.726
HOMA2-%B	0.130	0.140	0.087	0.363	0.080	0.419
HOMA2-%S	0.028	0.753	0.044	0.642	0.037	0.709
HOMA2-IR	−0.015	0.865	0.005	0.954	0.016	0.872

Model 1: unadjusted.
Model 2: adjusted for age, gender, and BMI.
Model 3: adjusted for age, gender, BMI, triglyceride, cholesterol, use of insulin, insulin secretagogues treatment, other antidiabetic medications, and duration of diabetes.

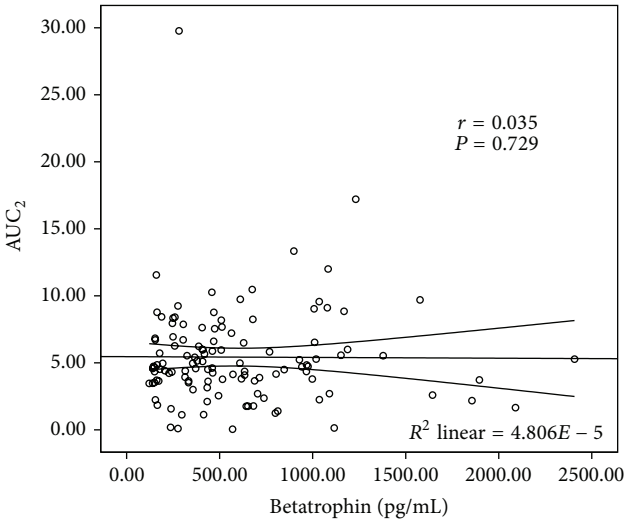
Table 4: Partial correlations between irisin levels and glucose-related variables in T2DM subjects.

Characteristics	Model 1		Model 2		Model 3	
	r	P-value	Partial r	P-value	Partial r	P-value
HbA1c	0.062	0.481	0.083	0.383	0.080	0.415
FBG	−0.166	0.053	−0.044	0.646	−0.069	0.486
Fasting insulin	0.031	0.720	0.012	0.899	0.031	0.751
Fasting C-peptide	−0.013	0.886	−0.025	0.796	−0.025	0.803
$AUC_{0.5}$	−0.091	0.318	−0.071	0.455	−0.067	0.497
AUC_1	−0.085	0.349	−0.069	0.470	−0.066	0.505
AUC_2	−0.065	0.481	−0.047	0.620	−0.051	0.607
HOMA2-%B	−0.050	0.569	−0.061	0.520	−0.021	0.828
HOMA2-%S	**0.243**	**0.005**	**0.225**	**0.017**	**0.218**	**0.026**
HOMA2-IR	−0.066	0.454	−0.031	0.745	−0.045	0.647

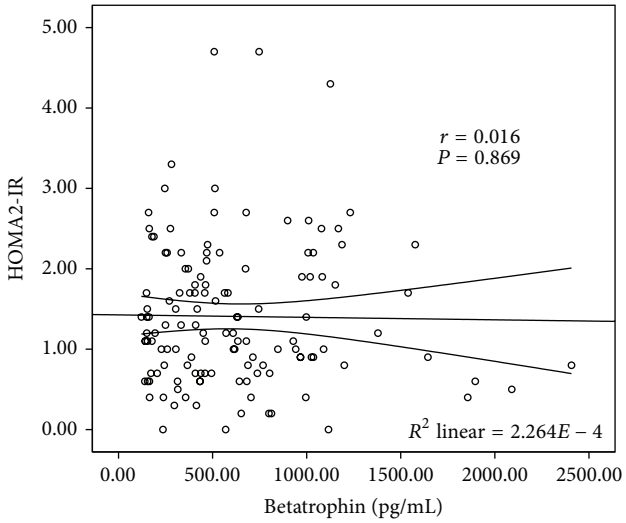
Model 1: unadjusted.
Model 2: adjusted for age, gender, and BMI.
Model 3: adjusted for age, gender, BMI, triglyceride, cholesterol, use of insulin, insulin secretagogues treatment, other antidiabetic medications, and duration of diabetes.



(a) Correlation between betatrophin levels and fasting C-peptide in T2DM subjects

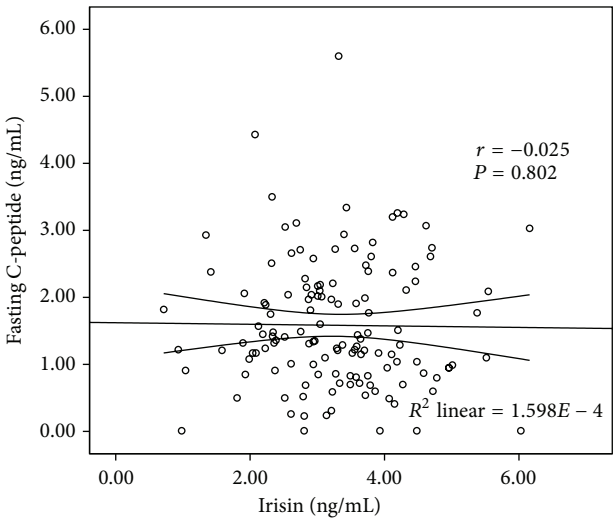


(b) Correlation between betatrophin levels and AUC_2 in T2DM subjects

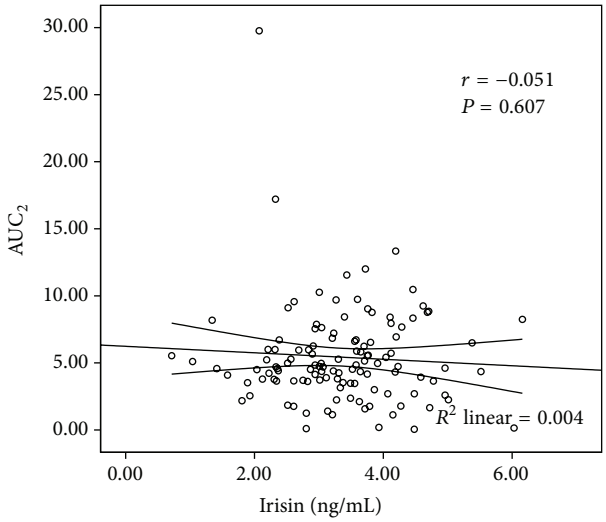


(c) Correlation between betatrophin levels and Homa2-IR in T2DM subjects

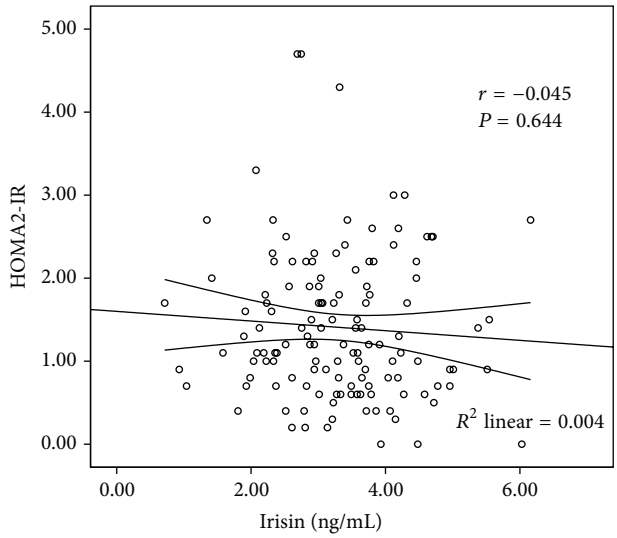
Figure 1: Correlation between betatrophin levels and glucose-related variables in T2DM subjects.



(a) Correlation between irisin levels and fasting C-peptide in T2DM subjects



(b) Correlation between irisin levels and AUC_2 in T2DM subjects



(c) Correlation between irisin levels and HOMA2-IR in T2DM subjects

Figure 2: Correlation between irisin levels and glucose-related variables in T2DM subjects.

resistance (HOMA-IR, quantitative insulin sensitivity check index (QUICKI), the Gutt insulin sensitivity index (ISI_G) and the Matsuda insulin sensitivity index (ISI_M)); however, correlation between betatrophin and HOMA2-IR or HOMA2-%S is not observed in our population. These inconformities might be due to the different population we chosen. Different from the newly diagnosed T2DM patients, our subjects had a mean diabetic history of 9 years and are under antidiabetic treatment. Medications such as metformin, TZD, and exogenous insulin could potentially alter the degree of insulin resistance and therefore affect the relevance between betatrophin and insulin resistance.

The ability of irisin to induce browning of WAT is of considerable interest for research on obesity, diabetes, and general metabolism [21–23]. Therefore, since its discovery, numerous studies have reported on the association of irisin with metabolic diseases in human cohorts. Recently, a meta-analysis revealed that significantly lower levels of circulating irisin were present in patients with T2DM, indicating the possible important role of irisin in regulating glucose homeostasis [24]. Similar phenomenon was also observed in this study as expected. However, the correlation between irisin and pancreatic β-cell function remains unclear. A recently published article found that serum irisin levels were closely related to HOMA-β in NGT subjects, but they did not observe a similar relationship in diabetes subjects. So researchers suggested that irisin might promote insulin secretion by increasing the proliferation of β-cells and the absence of such a correlation in T2DM patients might be due to the limited sample size ($n = 60$) [9]. However, by enlarging the sample size and applying the AUC of C-peptide to assess pancreatic β-cell function, which is the gold

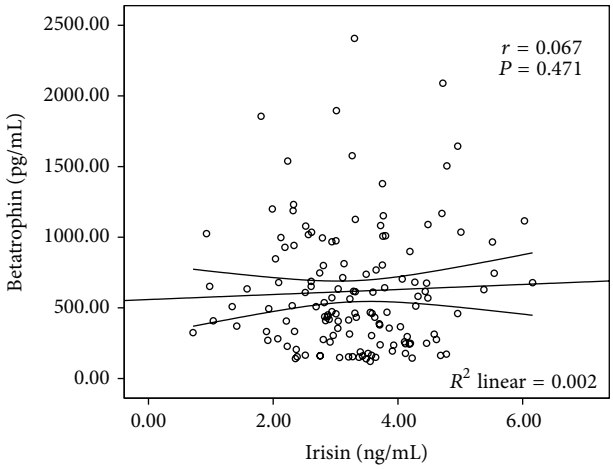


Figure 3: Correlation between irisin and betatrophin levels in T2DM subjects.

standard measure so far [11], our study still could not find a significant association between irisin levels and pancreatic β-cell function. So we speculated that irisin may correlate with pancreatic β-cell function in NGT subjects, but after a person has gotten diabetes, the circulating irisin levels will not have enough capacity to significantly affect pancreatic β-cell function in such a disorder internal environment. Besides, contradictory to the results of Zhang et al. [10], we found betatrophin and irisin levels lacked a significant correlation in T2DM subjects, which might remind us to be very careful when applying the results from animal models to humans.

Additionally, similar to what has been reported [25], we found that irisin is positively associated with insulin sensitivity (HOMA2-%S), even after controlling multiple covariates such as age, gender, BMI, blood lipids, antidiabetic medications, and the duration of diabetes. However the previous findings about the association between irisin and insulin resistance are controversial in different researches. Park et al. [23] reported that circulating irisin levels were associated positively with HOMA-IR. However, Al-Daghri et al. [26] conducted a study in a cohort of 153 Saudi Arabian children and found in girls, but not in boys, HOMA-IR correlated negatively with irisin levels. These above results suggested the relationship between irisin and insulin resistance need to be further warranted, especially in T2DM subjects.

All our data indicates that (1) betatrophin may not control pancreatic β-cell expansion or regulate pancreatic β-cell function in T2DM patients; (2) irisin may not promote pancreatic β-cell proliferation or regulate pancreatic β-cell function through promoting the expression of betatrophin in humans; (3) the beneficial effect of irisin on glucose homeostasis may be due to other mechanisms, such as inducing browning of WAT [8, 10], rather than the direct effect on β-cell function.

The strengths of our study were that we used AUC of C-peptide during 75-g OGTT to reflect pancreatic β-cell function, which is the gold standard measure of β-cell function [11]. Both the acute insulin response ($AUC_{0-0.5}$ and AUC_{0-1}) and the whole β-cell capacity (AUC_{0-2}) were analysed in our study. Meanwhile, our study has some limitations. First, a cross-sectional study could not infer causality between these two hormones and β-cell function. Second, we included previously diagnosed T2DM patients; the medication history, especially insulin usage, may affect results. However, the indexes of β-cell function and insulin resistance were calculated based on C-peptide instead of insulin, which ensured the accuracy of results as far as possible. Third, as betatrophin and irisin levels are not just related to β-cells but also adipocytes and other cells, betatrophin and irisin levels may correlate with other adipokines or cytokines such as leptin [27, 28]. These factors were not adjusted in our model and thus might influence our results. Finally, HOMA2-%S and HOMA2-IR could not accurately reflect insulin sensitivity and insulin resistance. The relationships between these two hormones and insulin resistance need further investigation.

5. Conclusion

In conclusion, we have found that the circulating levels of betatrophin were significantly elevated, while irisin levels were significantly decreased, in patients with T2DM compared to NGT subjects. However, circulating betatrophin levels were not associated with β-cell function and insulin resistance in previously diagnosed T2DM patients, while irisin correlated positively with insulin sensitivity but is not associated with β-cell function-related variables. Considering the complexity of the mechanisms of betatrophin and irisin functioning, we cannot exclude the possibility that the betatrophin and irisin levels might be associated with other unknown intermediate factors, which may affect β-cell function and insulin resistance. Further studies, especially those with histology evidences, are needed to demonstrate the associations of betatrophin and irisin with β-cell function in diabetic subjects.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Lingshu Wang and Jun Song contributed equally to this work.

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Research Article

Relationship of Hemoglobin A1c with β Cell Function and Insulin Resistance in Newly Diagnosed and Drug Naive Type 2 Diabetes Patients

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Objective. To investigate changes in the glycated hemoglobin A1c (A1c) level and those in β cell function and insulin resistance in newly diagnosed and drug naive type 2 diabetes patients and to evaluate the relationship between them. *Design and Methods*. A total of 818 newly diagnosed diabetic individuals who were ≥40 years of age were recruited. The subjects were grouped by A1c values (<6.5%, 6.5–7%, 7-8%, 8-9%, and ≥9%). The homeostasis model assessment (HOMA) was used to evaluate pancreatic β cell function (HOMA-β) and insulin resistance (HOMA-IR). ANOVA, t-tests, and binary logistic regression analysis were used for data analysis. *Results*. Compared with subjects with A1c values <6.5%, individuals with an A1c of 6.5–7% exhibited an increased HOMA-β index. However, the HOMA-β index was significantly decreased at A1c values ≥7% and further decreased by 9.3% and by 23.7%, respectively, at A1c values of 7-8% and 8-9%. As A1c increased to ≥9%, a 62% reduction in β cell function was observed, independently of age, gender, body mass index (BMI), blood pressure (BP), blood lipids, and hepatic enzyme levels. Meanwhile, insulin resistance was significantly increased with an increase in A1c values. *Conclusions*. Elevated A1c values (≥7%) were associated with substantial reductions in β cell function.

1. Introduction

The main pathophysiological defects responsible for type 2 diabetes mellitus (T2DM) include β cell dysfunction and decreased insulin sensitivity [1]. In the presence of insulin resistance, progressive loss of β cell function is a crucial defect [2]. Many factors including hyperglycemia and elevated free fatty acid accelerate β cell deterioration [3]. Accumulating evidence has shown that sustained hyperglycemia is deleterious to β cell function. The hemoglobin A1c (A1c) value is an integrated measure of mean glycemia over the preceding 8–12 weeks and is considered the "gold standard" for monitoring metabolic control in subjects with diabetes [4]. It has been reported that an increase in the A1c level is usually accompanied by a decline in pancreatic β cell function. However, little is known about the relationship between the A1c level and β cell function, especially in newly diagnosed and drug naive type 2 diabetic patients. This study was performed to investigate the changes in A1c along with β cell function and insulin resistance in newly diagnosed and drug naive type 2 diabetic patients and to evaluate the relationship between them.

2. Materials and Methods

2.1. Ethics Statement. The present work consists of one part of the baseline survey from the Risk Evaluation of cAncers in Chinese diabeTic Individuals: a lONgitudinal (REACTION) study, which was conducted among 259,657 adults, aged 40 years and older, in 25 communities across mainland China from 2011 to 2012 [5–8]. This study was approved by the Ruijin Hospital Ethics Committee of the Shanghai Jiao Tong University School of Medicine. Written informed consent was obtained from the study participants.

2.2. Study Population. A total of 10,028 subjects were recruited (40 years of age and older) in Shandong province from January to April 2012. Based on previous medical histories and OGTT, we selected 818 newly diagnosed and drug naive type 2 diabetes patients. The exclusion criteria consisted of (1) previously diagnosed hepatic disease, including fatty liver, liver cirrhosis, and autoimmune hepatitis; (2) previously diagnosed diabetes; and (3) any malignant disease. A total of 818 subjects (508 women) were eligible for the analysis.

2.3. Data Collection. The demographic characteristics, lifestyle, and previous medical histories were obtained by trained investigators through a standard questionnaire. All subjects underwent a baseline evaluation including body mass index (BMI), waist circumference (WC), and blood pressure (BP). Laboratory evaluations of fasting blood glucose (FBG), fasting insulin, cholesterol, triglyceride, ALT, and AST levels were also performed. OGTTs were conducted in all patients, using a glucose load containing the equivalent of 75 g of anhydrous glucose dissolved in water. The A1c level was measured by high-performance liquid chromatography (VARIANT II and D-10 Systems, BIO-RAD, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as follows: fasting insulin concentration (mIU/L) × FBG concentration (mmol/L)/22.5. The HOMA-β index was calculated as follows: 20 $*$ fasting insulin concentration (mIU/L)/(FBG concentration (mmol/L) − 3.5) [9].

2.4. Definition. Diabetic patients who were diagnosed based on the 1999 World Health Organization (WHO) criteria (FBG ≥ 126 mg/dL (7.0 mmol/L) and/or 2hPG ≥ 200 mg/dL (11.1 mmol/L)) [10] were identified after OGTTs. To explore the association between A1c and insulin resistance/β cell function, we divided the subjects into the following five groups according to the A1c values: <6.5%, 6.5–7%, 7-8%, 8-9%, and ≥9%.

2.5. Statistical Analysis. Continuous variables with a normal distribution are expressed as the means ± standard deviation (SD), and variables with a nonnormal distribution are presented as medians (interquartile range). Categorical variables are presented as numbers (%). Between-group differences were evaluated with ANOVA. Binary logistic regression analysis was used to estimate the association between A1c levels and β cell function/insulin resistance in three models. The following three models were constructed: Model 1 = not adjusted; Model 2 = adjusted for age, gender, BMI, and WC; Model 3 = Model 2 plus systolic BP, diastolic BP, cholesterol, triglycerides, ALT, and AST values. A value of $P < 0.05$ was considered statistically significant. The data were analyzed using the SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Characteristics of Study Participants Grouped by A1c Category. We recruited a total of 818 newly diagnosed and drug naive diabetic subjects, including 508 females and 310 males with an average age of 60.4 ± 9.6 years. The subjects were divided into five groups according to their A1c levels. As shown in Table 1, no difference in gender, systolic BP, cholesterol, LDL-C, and AST levels were observed between groups. Individuals with an A1c of 7-8% were more likely to be older and stronger and have a higher diastolic BP than those with an A1c < 6.5%.

3.2. β Cell Function and Insulin Resistance Changes in Different A1c Groups. We used the HOMA-β index to assess β cell function. As shown in Figure 1(a), compared to subjects with an A1c < 6.5%, individuals with an A1c of 6.5–7% exhibited increased β cell function. By contrast, the HOMA-β index was significantly decreased in individuals with an A1c ≥ 7%. As A1c increased to ≥9%, a 62% reduction in β cell function was observed. We further compared β cell function at different A1c levels in male and female subjects. Impaired β cell function was observed in subjects with an A1c ≥ 8% in both male and female patients; the values were decreased by 26% and 48%, respectively. Furthermore, the HOMA-β index values in female patients were significantly higher than those in male patients with an A1c ≥ 9%.

We further used the HOMA-IR index to assess insulin resistance in different A1c groups (Figure 1(b)). Insulin resistance increased significantly with increasing A1c levels. Compared with the A1c < 6.5% group, insulin resistance increased by 9%, 14%, 18%, and 29% in individuals with A1c values of 6.5–7%, 7-8%, 8-9%, and ≥9%, respectively. In male patients, insulin resistance was significantly higher in individuals with an A1c ≥ 7% than in individuals with an A1c < 6.5%, while in female patients, the HOMA-IR index was increased only in individuals with an A1c ≥ 9%. No significant difference was observed between male and female patients.

3.3. Binary Logistic Regression Analysis. As shown in Table 3, we analyzed the association between increased A1c levels and impaired β cell function using three models. We found that patients with A1c values of 8%-9% and ≥9% had a significantly decreased β cell function (odds ratio (OR) = 2.45 and 15.36, resp.). After adjusting for age, gender, BMI, and WC, these two groups still presented increased ORs (3.69 and 22.08, resp.). After further adjusting for systolic BP, diastolic BP, cholesterol, triglycerides, ALT, and AST, the patients with A1c levels of 8%-9% and ≥9% also showed an increased risk of impaired β cell function (OR = 4.19 and 28.51, resp.).

Table 1: Characteristic of study participants grouped by A1c category.

Characteristics	Group 1	Group 2	Group 3	Group 4	Group 5	Total
N	311	156	172	67	112	818
Female (%)	198 (63.7%)	103 (66.0%)	109 (63.4%)	38 (56.7%)	60 (53.6%)	508 (62.1%)
Age (years)	59.44 ± 9.91	61.7 ± 9.69	61.56 ± 9.23*	61.76 ± 8.72	58.55 ± 8.77	60.39 ± 9.54
BMI (kg/m^2)	26.44 ± 3.51	27.72 ± 3.19**	27.99 ± 3.31***	27.56 ± 3.38	26.36 ± 2.86	27.09 ± 3.39
Wc (cm)	87.7 ± 10.16	91.53 ± 8.88***	91.51 ± 10.25***	91.63 ± 10.84**	89.56 ± 8.71	89.81 ± 09.95
Systolic BP (mmHg)	146.15 ± 20.63	146.56 ± 19.42	145.76 ± 19.85	146.31 ± 18.83	147.99 ± 21.11	146.4 ± 20.13
Diastolic BP (mmHg)	83.96 ± 12.49	82.4 ± 11.76	81.02 ± 11.34**	80.31 ± 10.69*	84.22 ± 12.38	82.78 ± 12.02
FBG (mmol/L)	7.47 ± 1.58	7.4 ± 0.91	7.96 ± 1.14**	8.86 ± 1.73***	12.54 ± 3.38***	8.37 ± 2.47
Fasting insulin (uU/mL)	9.7 (7–13.95)	11.3 (7.8–15.8)	9.7 (6.93–14.6)*	9.9 (7–14.8)	7.4 (5.23–11.1)**	9.85 (6.9–14)
A1c (%)	5.96 ± 0.34	6.7 ± 0.14***	7.39 ± 0.3***	8.33 ± 0.28***	10.86 ± 1.75***	7.27 ± 1.75
Cholesterol (mmol/L)	5.51 ± 1.14	5.68 ± 1.01	5.6 ± 0.97	5.58 ± 0.95	5.76 ± 1.42	5.6 ± 1.11
Triglycerides (mmol/L)	1.42 (1.01–2.1)	1.61 (1.19–2.27)	1.73 (1.26–2.47)	1.91 (1.3–2.8)*	1.81 (1.28–2.36)***	1.59 (1.12–2.27)
LDL-C (mmol/L)	3.3 ± 0.89	3.45 ± 0.84	3.4 ± 0.83	3.32 ± 0.83	3.64 ± 0.95	3.4 ± 0.88
HDL-C (mmol/L)	1.55 ± 0.39	1.44 ± 0.27**	1.36 ± 0.28***	1.34 ± 0.24***	1.48 ± 0.46	1.46 ± 0.36
ALT (U/L)	13.67 ± 9.75	15.58 ± 11.06**	16.94 ± 13.29	16.19 ± 9.89	15.71 ± 10.08	15.21 ± 10.94
AST (U/L)	20.74 ± 9.32	21.13 ± 9.03	22.434 ± 9.67	21.64 ± 11.32	19.27 ± 8.97	21.03 ± 9.49

Data are mean ± SD or median (interquartile range) or number (%). BMI, body mass index; WC, waist circumference; BP, blood pressure; FBG, fasting blood glucose; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase. $^{*}P < 0.05$ compared with Group 1; $^{**}P < 0.01$ compared with Group 1; $^{***}P < 0.01$ compared with Group 1.

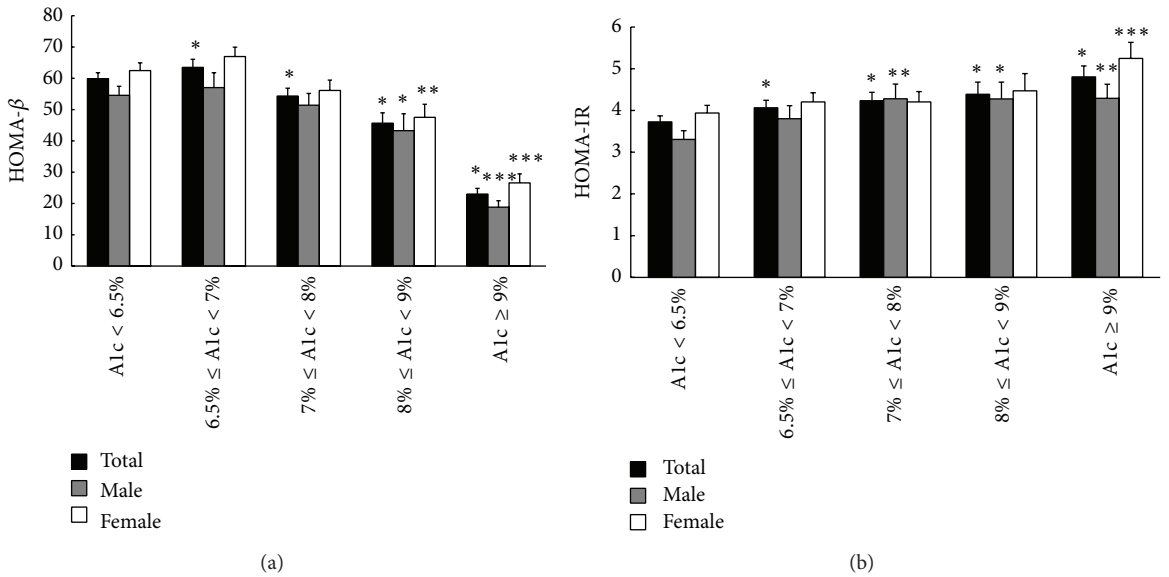

Figure 1: (a) β cell function with different A1c groups (HOMA-β). (b) Insulin resistance with different A1c groups (HOMA-IR).

Similarly, we analyzed the association between increased A1c levels and insulin resistance using the three models (Table 2). As expected, the A1c value was significantly increased with increased insulin resistance. In Model 1, the patients with A1c values of 8%-9% and ≥9% had a significantly increased risk of insulin resistance (odds ratio (OR) = 1.89 and 1.85, resp.). After adjusting for age, gender, BMI, and WC, group 5, with an A1c ≥ 9%, also presented an increased OR (2.16). After further adjusting for systolic BP, diastolic BP, cholesterol, triglycerides, ALT, and AST, patients with an A1c value of 9% also showed an increased risk of insulin resistance (OR = 2.04, $P = 0.014$).

4. Discussion

The prevalence of diabetes has increased significantly in recent decades and is now reaching epidemic proportions in China. The most recent national survey in 2013 reported that the prevalence of diabetes and prediabetes in Chinese adults was 11.6% and 50.1%, respectively [11]. The main pathophysiological defects responsible for type 2 diabetes mellitus (T2DM) are β cell dysfunction and decreased insulin sensitivity. Pancreatic β cell dysfunction plays a major role in determining dysglycemia from the onset of diabetes [12, 13]. Studies from UKPDS have documented a reduction in β cell

Table 2: Logistic regression analysis of the association between different A1c groups and insulin resistance.

Characteristics	Model 1		Model 2		Model 3	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
A1c groups						
Group 1	1 (reference)		1 (reference)		1 (reference)	
Group 2	1.30 (0.82–2.05)	0.264	0.95 (0.57–1.57)	0.834	0.91 (0.54–1.53)	0.708
Group 3	1.29 (0.83–2.00)	0.266	1.03 (0.64–1.68)	0.892	1.01 (0.61–1.68)	0.969
Group 4	1.89 (1.06–3.37)	**0.032**	1.66 (0.86–3.21)	0.134	1.55 (0.76–3.17)	0.226
Group 5	1.85 (1.13–3.02)	**0.014**	2.16 (1.26–3.69)	0.005	2.04 (1.16–3.61)	**0.014**
Female	—		1.79 (1.22–2.63)	**0.003**	2.30 (1.50–3.51)	<**0.001**
Age (years)	—		0.98 (0.96–0.99)	**0.010**	1.00 (0.98–1.02)	0.882
BMI (kg/m^2)	—		1.13 (1.05–1.21)	**0.001**	1.11 (1.03–1.19)	**0.007**
WC (cm)	—		1.05 (1.02–1.07)	<**0.001**	1.05 (1.02–1.07)	**0.001**
Systolic BP (mmHg)	—		—		1.00 (0.98–1.02)	0.772
Diastolic BP (mmHg)	—		—		1.03 (1.01–1.06)	**0.001**
Cholesterol (mmol/L)	—		—		0.97 (0.80–1.19)	0.787
Triglycerides (mmol/L)	—		—		1.17 (1.02–1.33)	**0.021**
AST (U/L)	—		—		1.01 (0.98–1.04)	0.646
ALT (U/L)	—		—		1.02 (0.99–1.05)	0.118

Model 1: unadjusted. Model 2: adjusted for age, gender, BMI, and WC. Model 3: adjusted for age, gender, BMI, WC, Systolic BP, Diastolic BP, Cholesterol, Triglycerides, AST, and ALT.

Table 3: Logistic regression analysis of the association between different A1c groups and impaired β cell function.

Characteristics	Model 1		Model 2		Model 3	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
A1c groups						
Group 1	1 (reference)		1 (reference)		1 (reference)	
Group 2	0.80 (0.45–1.40)	0.427	1.22 (0.65–2.28)	0.536	1.33 (0.70–2.53)	0.377
Group 3	1.47 (0.92–2.37)	0.111	2.47 (1.42–4.27)	**0.001**	2.79 (1.58–4.94)	<**0.001**
Group 4	2.45 (1.35–4.48)	**0.003**	3.69 (1.82–7.48)	<**0.001**	4.19 (1.99–8.79)	<**0.001**
Group 5	15.36 (9.05–26.07)	**0.014**	22.08 (11.86–41.12)	<**0.001**	28.51 (14.53–55.95)	<**0.001**
Female	—		0.38 (0.25–0.59)	<**0.001**	0.39 (0.25–0.61)	<**0.001**
Age (years)	—		0.99 (0.97–1.01)	0.356	0.99 (0.97–1.02)	0.398
BMI (kg/m^2)	—		0.76 (0.70–0.83)	<**0.001**	0.77 (0.70–0.84)	<**0.001**
WC (cm)	—		0.99 (0.96–1.02)	0.405	0.99 (0.96–1.02)	0.455
Systolic BP	—		—		0.99 (0.98–1.01)	0.290
Diastolic BP	—		—		1.01 (0.99–1.03)	0.545
Cholesterol	—		—		0.96 (0.77–1.19)	0.699
Triglycerides	—		—		0.86 (0.73–1.02)	0.083
AST	—		—		1.03 (0.99–1.06)	0.155
ALT	—		—		0.98 (0.95–1.01)	0.137

Model 1: unadjusted. Model 2: adjusted for age, gender, BMI, and WC. Model 3: adjusted for age, gender, BMI, WC, Systolic BP, Diastolic BP, Cholesterol, Triglycerides, AST, and ALT.

function of up to 50% at the time of diagnosis, and this value gradually increases with the progression of diabetes [14]. Increasing evidence suggests that β cell function protection should be a priority starting at the onset of diabetes.

The A1c is an integrated measure of mean glycemia over the preceding 8–12 weeks, and the ADA has recommended the A1c value as a diabetes diagnosis standard [15]. Previous studies have shown that an increase in the A1c level is usually accompanied by a decline in pancreatic β cell function. However, whether this trend is present in newly diagnosed and drug naive diabetes patients remains unclear. Moreover, β cell function changes at different A1c levels, and these changes in newly diagnosed T2DM patients have not been characterized. In this study, we divided the subjects into five

groups based on their A1c levels and compared β cell function and insulin resistance at different A1c levels. We found that individuals with an A1c of 6.5–7% exhibited an increased HOMA-β index compared with subjects who had an A1c < 6.5%, indicating that a slight increase in the A1c level may induce increases in insulin secretion, which occur to compensate for rising insulin resistance. However, the HOMA-β index was significantly decreased in patients with A1c levels ≥ 7%; it was decreased by 9.3% in patients with an A1c of 7-8% and by 23.7% in patients with an A1c 8-9%. As the A1c level increased to ≥9%, a 62% reduction in β cell function was observed, which suggests that poor glycemic control may contribute to the decrease in β cell function. These results are consistent with a previous study [16]. Because the A1c value was significantly correlated with β cell function in newly diagnosed and drug naive type 2 diabetes patients, our present finding could have potentially important clinical implications. Attention should be focused on the A1c value to protect β cell function in diabetic patients. The underlying mechanisms of this process may be found in diabetic rodent studies. Sustained hyperglycaemia damages β cell function via several mechanisms such as an increase in oxidative stress, activation of the MAPK pathway, and reduction of the pancreatic and duodenal homeobox factor-1 (PDX-1) function [17, 18].

In addition to hyperglycemia, the traditional risk factors of impaired β cell function include age, obesity, hypertension, ALT, AST, and dyslipidemia. We adjusted for age, gender, BMI, systolic BP, diastolic BP, ALT, and AST, as well as WC, cholesterol, and triglyceride levels to ensure that our results were more reliable. After adjusting for the above risk factors, the A1c value was still significantly associated with impaired β cell function in patients with A1c levels ≥ 7%; no changes were observed in individuals with an A1c < 7%. To date, no study has detailed insulin sensitivity in Chinese subjects with newly presented type 2 diabetes mellitus stratified by A1c levels. Our study also demonstrated that insulin resistance increased with increasing A1c levels, compared to individuals with an A1c < 6.5%.

Our study also has some limitations. First, our study included only middle-aged and elderly Chinese subjects; therefore, the results might not be applicable to subjects of different ages or ethnicities. Second, we used the HOMA-IR index to evaluate insulin resistance instead of the "gold standard" (euglycemic-hyperinsulinemic clamp techniques). The HOMA-IR index is a mathematical model of the fasting state, and it represents hepatic insulin resistance only; therefore, it cannot reflect insulin resistance accurately. Third, this is only a cross-sectional study. Long-term prospective studies are needed to clarify the association between the changes in β cell function and A1c levels during the development of diabetes.

5. Conclusions

Elevated A1c levels (≥7%) were associated with substantial reductions in β cell function. The A1c value could be used as simple and practical index to evaluate β cell function and direct clinical treatment in newly diagnosed and drug naive type 2 diabetes patients.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Xinguo Hou and Jinbo Liu contributed equally to this work.

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Clinical Study

Effects of Liraglutide Combined with Short-Term Continuous Subcutaneous Insulin Infusion on Glycemic Control and Beta Cell Function in Patients with Newly Diagnosed Type 2 Diabetes Mellitus: A Pilot Study

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The objective of this paper is to investigate the effects of liraglutide in combination with short-term continuous subcutaneous insulin infusion (CSII) therapy on glycemic control and beta cell function in patients with newly diagnosed type 2 diabetes mellitus (T2DM). Thirty-nine eligible newly diagnosed T2DM patients were recruited and randomized to receive either of two therapies: short-term CSII alone (CSII alone group) or CSII in combination with liraglutide (CSII + Lira group) for 12 weeks. Blood glucose control, homeostasis model assessment (HOMA) indices, and acute insulin response (AIR) were compared between the two groups. The patients in CSII + Lira group achieved euglycemia with equivalent insulin dosage in shorter time (1 (0) versus 2 (3) days, $P = 0.039$). HbA1c at the end of study was comparable between two groups (6.3 ± 0.7% versus 6.0 ± 0.5%, for CSII alone group and CSII + Lira group, resp., $P = 0.325$). The increment of AIR was higher in CSII + Lira group (177.58 (351.57) μU·min/mL versus 58.15 (51.30) μU·min/mL, $P < 0.001$). However, after stopping liraglutide, its effect on beta cell function disappeared completely. Liraglutide combined with short-term CSII was effective in further improving beta cell function, but the beneficial effects did not sustain after suspension of the therapy.

1. Introduction

Diabetes mellitus is the most common metabolic disease and becomes a heavy burden of public health systems. In China, the prevalence of diabetes and prediabetes in adults was 11.6% and 50.1%, respectively [1]. Deterioration of beta cell function and insulin resistance are two fundamental pathophysiologic defects of type 2 diabetes mellitus (T2DM). It has been proven that at the time when T2DM was established, the loss of beta cell function was shown to reduce by 50% and this decline of beta cell function progressed over time although traditional antihyperglycemic therapy had been applied [2]. In order to postpone the progress of disease, new therapies are required to persistently act on beta cell failure and insulin resistance.

In our previous studies, intensive insulin interventions, especially continuous subcutaneous insulin infusion (CSII), induced near-normoglycemia over 1 year without antihyperglycemic agents in nearly half of the patients with newly diagnosed T2DM with favorable recovery of beta cell function [3, 4]. The reason for glycemic remission in these patients was considered to be alleviation of glucotoxicity, lipotoxicity, and insulin resistance [5, 6]. However, the therapy, which lasted for only 2-3 weeks, had its limitations in covering the multiple pathophysiological defects in the long term. In another trial investigating the effect of combination of metformin or rosiglitazone with CSII, the combination of metformin for 3 months had better effects on insulin secretion function measured by acute insulin response (AIR) and HOMA-B while the combination with rosiglitazone better improved muscle insulin resistance [7]. Since the two medicines used in that study mainly were targeted at insulin resistance, it would be of great interest whether combining CSII with medicine

intervening beta cell failure, the critical pathophysiology mechanism of T2DM, might provide better clinical outcomes compared with short-term CSII alone.

Liraglutide, a glucagon-like peptide-1 (GLP-1) analog with a 97% homology with endogenous GLP-1, lowers blood glucose by enhancing glucose-dependent insulin secretion of beta cells and suppressing glucagon secretion of alpha cells [8]. In some rodent studies, liraglutide reduced beta cell apoptosis and promoted its proliferation, which might potentially modify the progression of T2DM [9, 10]. Moreover, liraglutide also reduced body weight in a dose dependent manner, ameliorated lipid profiles, lowered blood pressure [11], and reduced cardiovascular risk markers such as adipokines and proinflammatory factors [12], all of which are favorable in management of T2DM. We hypothesized that combining CSII with liraglutide might have better effects over CSII alone. Therefore, we conducted this randomized controlled trial investigating whether liraglutide in combination with short-term CSII therapy has better effect over CSII alone on beta cell function and sustained glycemic control.

2. Subjects and Methods

2.1. Subjects. Thirty-nine newly diagnosed T2DM patients diagnosed according to the 1999 World Health Organization diagnostic criteria [13], without previous usage of antihyperglycemic and antihyperlipidemic medication, were enrolled. The included patients were between 20 and 65 years of age and had a body mass index of 20–35 kg/m^2, with fasting plasma glucose (FPG) between 7.0 and 16.7 mmol/L. Patients were excluded if they had severe acute or severe chronic diabetic complications and severe intercurrent illness and were positive for autoimmune antibodies against islets or with a recent history of being treated with corticosteroid, immunosuppressing drugs, or cytotoxic drugs.

2.2. Study Design. All patients were admitted to the hospitals after a 3–5-day run-in period and assigned to one of the following two groups by sequentially opening sealed, opaque envelopes arranged in a computer-generated random order. During hospitalization, patients in CSII alone group received insulin aspart (NovoRapid, Novo Nordisk, Bagsværd, Denmark) or insulin lispro (Humalog, Eli Lilly, USA) with an insulin pump (MiniMed 712, Medtronic, Northridge, CA) as CSII therapy, while the CSII + Lira group received liraglutide (Victoza, Novo Nordisk, Bagsvaerd, Denmark) 0.6 mg per day in addition to aforementioned CSII regimen. The initial insulin dosage was 0.5–0.7 IU/kg/d, with the total daily dosage divided into 50/50 as basal and bolus infusion. In order to achieve euglycemia, basal rates and premeal boluses of insulin were adjusted every day according to capillary blood glucose values which were monitored at least 7 times per day. The glycemic goal was defined as fasting blood glucose less than 6.0 mmol/L and postprandial blood glucose less than 8.0 mmol/L. After the glycemic targets were achieved, CSII treatments were maintained for additional 14 days. After being discharged from the hospital, all patients were guided with diet and physical exercise. Patients in CSII + Lira group continued to use liraglutide 1.2 mg per day until the 12-week treatment period was finished.

All recruited patients provided written informed consent for participation, and the study protocol was approved by the Medical Research and Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China). This study is registered at ClinicalTrials.gov with trial registration identifier number of NCT01471808.

2.3. Measurements. Baseline anthropometric data such as blood pressure, height, weight, and waist and hip circumferences were measured, while fasting blood samples were collected for measurements of FPG and HbA1c. An intravenous glucose tolerance test (IVGTT) using 25 g of glucose (50 mL of 50% glucose) was conducted to assess AIR which was used to estimate the first-phase beta cell insulin secretion. Serum insulin levels before and 1, 2, 4, 6, and 10 min after glucose injection were measured, and AIR was calculated as the incremental trapezoidal area during the first 10 min of the IVGTT. PPG (after breakfast) levels were evaluated on the previous day. Homeostasis model assessment was used to estimate insulin resistance (HOMA-IR) and beta cell function (HOMA-B). HOMA-IR = FPG × fasting insulin/22.5. HOMA-B = 20 × fasting insulin/(FPG − 3.5). Daily insulin dosage of each patient was recorded. After CSII suspension, all baseline measurements were repeated at least 15 hours after cessation of insulin infusion and before liraglutide injection for CSII + Lira group. At the 12-week visit, the assessments were performed after 12 weeks of CSII suspension for CSII alone group or 7 days after liraglutide suspension.

3. Statistical Analyses

Data were analyzed with SPSS software for Windows version 16.0. Normally distributed data were presented as mean ± SD, and nonnormally distributed variables (triglyceride, AIR, HOMA-B, and HOMA-IR) were expressed as median (interquartile range). The differences of normally distributed data between two groups were compared by independent-sample t-tests, while the comparisons of nonnormally distributed variables were using Mann-Whitney U tests. Paired-sample t-tests or Wilcoxon signed ranks tests were performed to estimate the changes before and after intervention. The χ^2 tests were applied to analyze the differences of proportions. A 2-sided value of $P < 0.05$ was defined statistically significant.

4. Results

4.1. Baseline Characteristics. The enrolled patients were 45.91 ± 8.7 years in age, with a BMI of 25.7 ± 2.8 kg/m^2, FPG of 11.4 ± 3.2 mmol/L, PPG of 17.4 ± 5.9 mmol/L, and HbA1c of 10.7 ± 2.2%. They were assigned to CSII alone group ($n = 19$) and CSII + Lira group ($n = 20$) and finished CSII therapy. At the subsequent 12-week visit 8 patients (20.5%, 4 in CSII alone group, 4 in CSII + Lira group) dropped out due to withdrawal of consent. At baseline there were no significant differences in clinical characteristics, FPG, and HbA1c between two groups

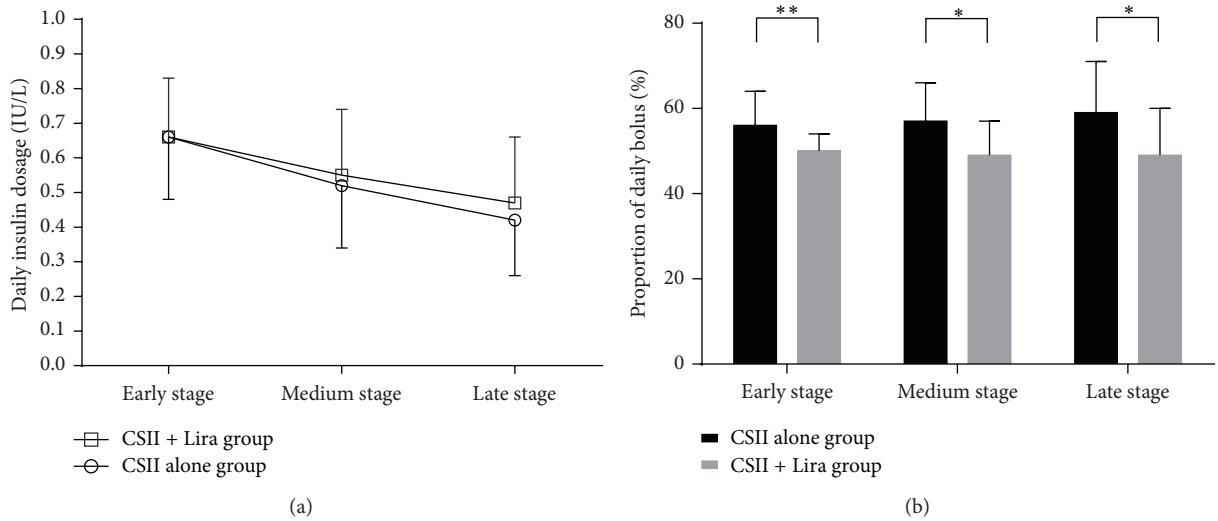

Figure 1: Daily insulin dosage and proportion of daily bolus of two groups. ((a) Daily insulin dosage, (b) proportion of daily bolus, $^{*}P < 0.05$, $^{**}P < 0.01$.)

except for PPG, which was slightly higher in CSII + Lira group (15.2 ± 6.1 mmol/L versus 14.4 ± 4.1 mmol/L, $P = 0.025$). Markers of beta cell function (AIR and HOMA-B) and insulin sensitivity (HOMA-IR) were also comparable (Table 1).

4.2. CSII Therapy. All patients achieved euglycemia in the first week of CSII treatments. Patients in CSII + Lira group reached target glycemic control in less time than those in CSII alone group (2 (3) days versus 1 (0) days, for CSII alone group and CSII + Lira group, resp., $P = 0.039$). After achieving euglycemia, daily insulin dosages decreased gradually. The 14 days of CSII for maintaining euglycemia was divided into three stages: early stage (days 1–5), medium stage (days 6–10), and late stage (days 11–14). Average daily insulin dosage was similar in both groups, while the proportions of daily bolus dosage in total daily insulin dosage were lower in CSII + Lira group throughout the CSII therapy (Figure 1).

4.3. Beta Cell Function. AIR was restored in all patients after short-term CSII therapy compared with baseline. At CSII suspension, AIR improved from −6.60 (26.2) μU·min/mL to 52.05 (100.55) μU·min/mL in CSII alone group and from −6.98 (21.71) μU·min/mL to 168.62 (350.95) μU·min/mL in CSII + Lira group. The increment of AIR was significantly higher in CSII + Lira group than that in CSII alone group (177.58 (351.57) μU·min/mL versus 58.15 (51.30) μU·min/mL, $P < 0.001$). However, after withdrawal of liraglutide after the 12-week treatment, the improvement in AIR rapidly disappeared in CSII + Lira group (168.62 (350.95) μU·min/mL versus 50.43 (70.40) μU·min/mL, for CSII suspension and 12-week visit, resp., $P < 0.001$). Therefore, AIR between two groups at the end of follow-up was similar ($P = 0.921$) (Figure 2(a)). In both groups, HOMA-B was ameliorated significantly after CSII treatment compared with baseline. Similar to AIR, HOMA-B in CSII + Lira group was higher than that in CSII alone group at the end of CSII (67.64 (46.31) versus 40.00 (35.53), $P = 0.007$), but the improvement was not sustained after stop of liraglutide at 12-week visit (41.28 (21.62), $P = 0.003$, compared with that after CSII suspension) and became

Table 1: Baseline characteristics of patients.

Characteristic	Group 1	Group 2	P value
Number	19	20	0.127
Gender (F/M)	4/15	5/15	0.770
Age (years)	42.1 ± 7.6	42.3 ± 9.9	0.127
Family history (with/without)	10/9	10/10	0.869
Blood pressure (mmHg)			
Systolic	116.5 ± 12.3	118.2 ± 11.7	0.914
Diastolic	74.4 ± 9.9	77.4 ± 12.0	0.372
Weight (kg)	71.6 ± 8.7	71.0 ± 8.2	0.441
BMI (kg/m^2)	25.5 ± 2.4	25.4 ± 2.8	0.743
Waist circumference (cm)	89.0 ± 6.9	89.4 ± 7.9	0.869
Waist to hip ratio	1.07 ± 0.62	0.94 ± 0.07	0.088
HbA1c (%)	10.2 ± 2.0	10.0 ± 2.1	0.862
FPG (mmol/L)	10.5 ± 3.3	10.4 ± 2.6	0.130
PPG (mmol/L)	15.2 ± 6.1	14.4 ± 4.1	0.025
Triglyceride (mmol/L)	1.80 (1.50)	1.43 (1.69)	0.899
CHOL (mmol/L)	5.1 ± 0.9	5.2 ± 1.1	0.408
LDL-c (mmol/L)	3.33 ± 0.83	3.52 ± 0.71	0.461
HDL-c (mmol/L)	1.18 ± 0.46	1.09 ± 0.19	0.099
AIR (μU·min/mL)	−6.60 (26.2)	−6.98 (21.71)	0.911
HOMA-IR	2.57 (2.78)	3.96 (2.71)	0.258
HOMA-B	20.48 (16.46)	20.16 (26.15)	0.584

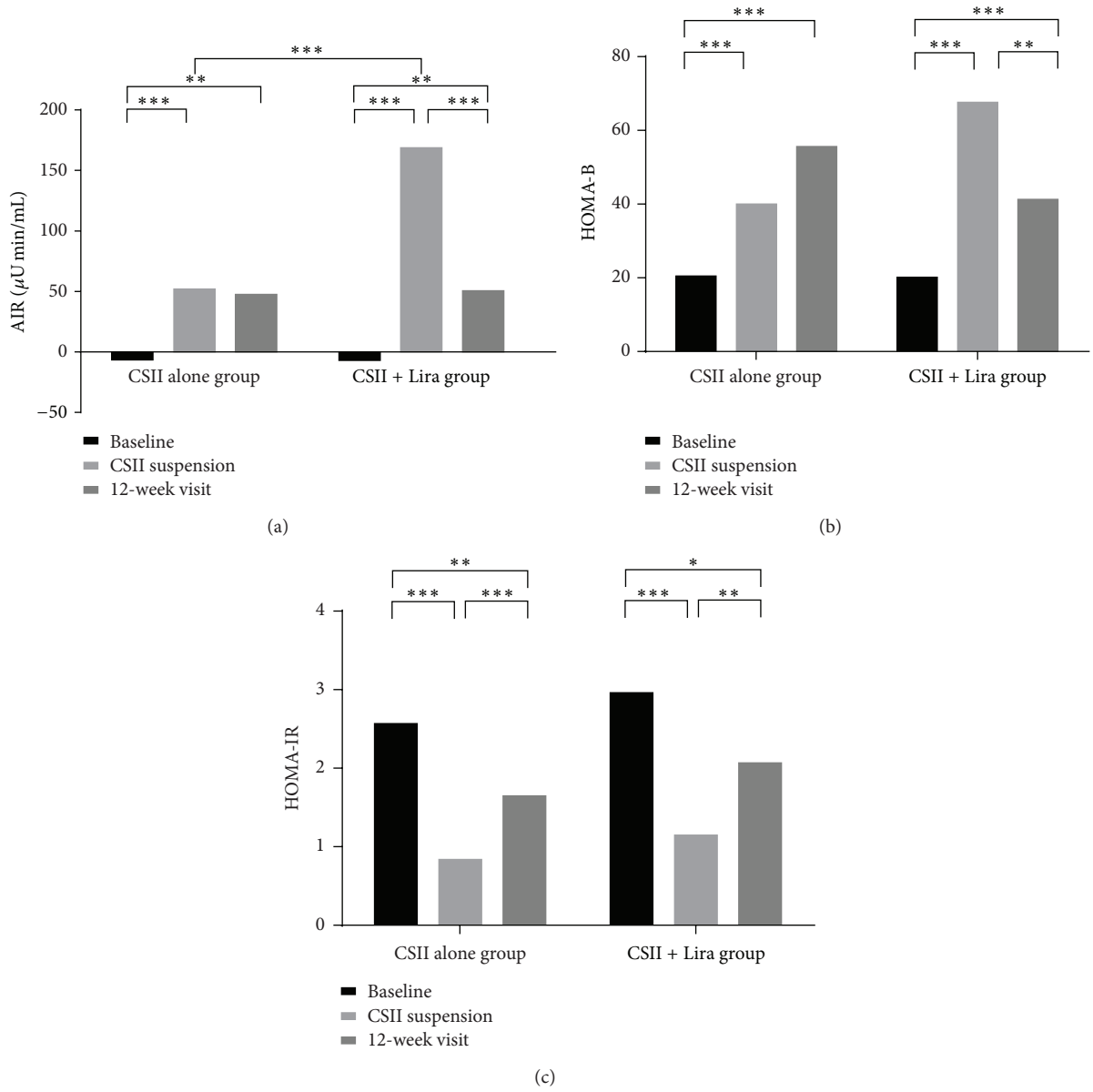

Figure 2: Beta cell function and insulin sensitivity of two groups, (a) AIR of baseline and after intervention, (b) HOMA-B of baseline and after intervention, and (c) HOMA-IR of baseline and after intervention, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

similar to CSII alone group (55.65 (56.27), $P = 0.110$) (Figure 2(b)).

4.4. Insulin Resistance. HOMA-IR decreased significantly after CSII compared with baseline in both groups. But at 12-week visit, HOMA-IR was significantly elevated from CSII suspension in both groups (Figure 2(c)).

4.5. Glycemic Control. HbA1c level was slightly lower in CSII + Lira group at the end of the 12-week follow-up compared with CSII alone group but did not reach statistical significance (6.0 ± 0.5% versus 6.3 ± 0.7%, $P = 0.325$), with similar proportions of patients who achieved HbA1c ≤ 6.5% (73% (11/15) versus 94% (15/16), for CSII alone group and CSII + Lira group, resp., $P = 0.146$) (Figure 3(a)). Considerable reduction in FPG and PPG from baseline was observed at CSII suspension. However, at 12-week visit there was a slight but statistically significant elevation of FPG in CSII + Lira group from CSII suspension (from 6.1 ± 0.9 mmol/L to 6.9 ± 1.1 mmol/L, $P = 0.01$), which was not seen in CSII alone group (Figure 3(a)). There was a tendency of higher hyperglycemia relapse rate (>7.0 mmol/L) in CSII + Lira group at 12-week visit (20% (3/15) versus 43.75% (7/16), for CSII alone group and CSII + Lira group, resp., $P = 0.208$) (Figure 3(b)).

4.6. Body Weight. At CSII suspension, certain body weight loss was recorded in both groups (−1.6 ± 2.0 kg versus −1.2 ± 2.3 kg, for CSII alone group and CSII + Lira group, resp.,

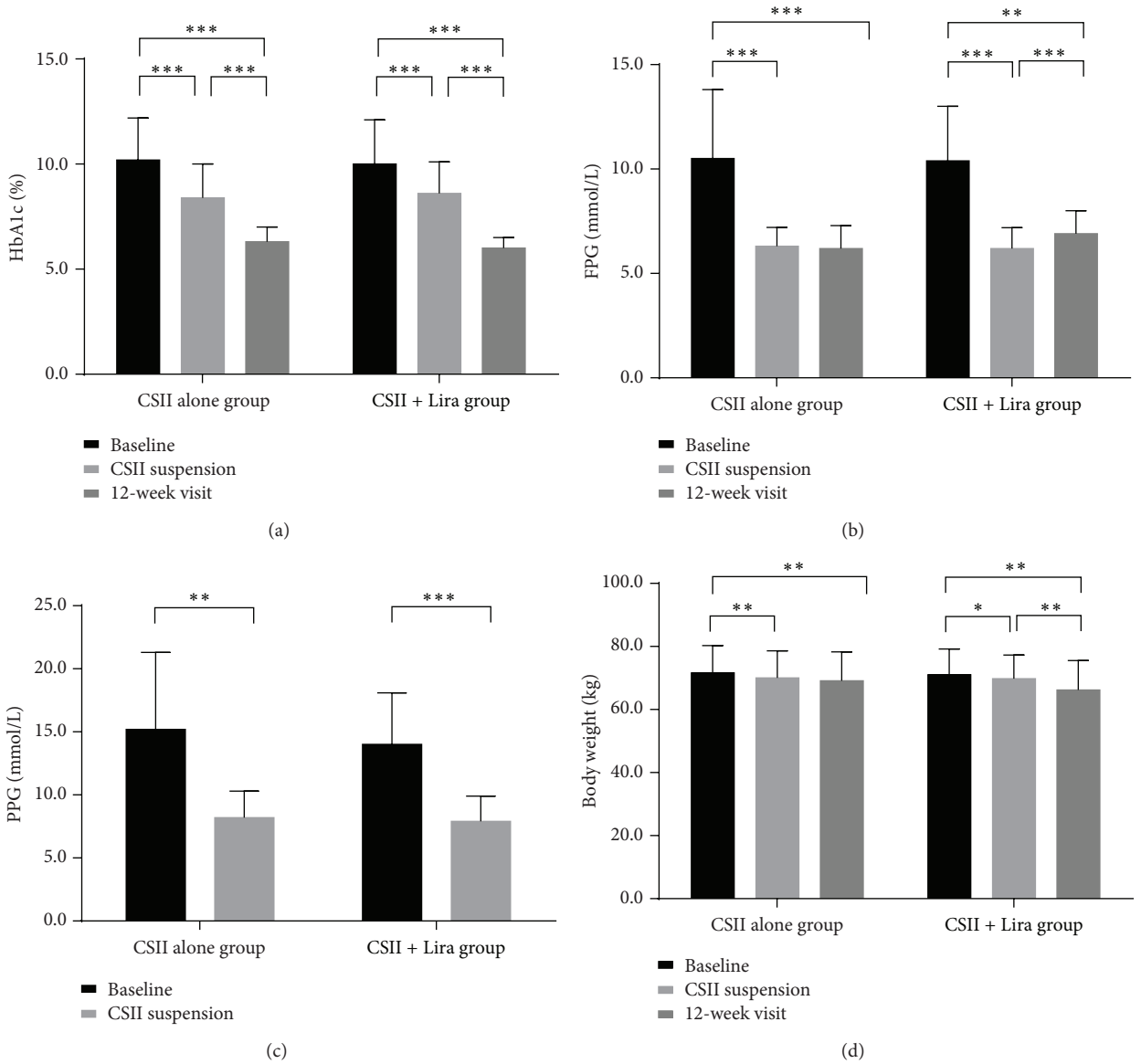

Figure 3: Glycemic control and body weight of two groups. (a) HbA1c of baseline and after interventions, (b) FPG of baseline and after interventions, (c) PPG of baseline and after CSII suspension, and (d) body weight of baseline and after intervention, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

$P = 0.574$). Continuous decline of body weight during the 12-week visit was recorded in CSII + Lira group (69.8±7.5 kg versus 66.2 ± 9.3 kg, for CSII suspension and 12-week visit, resp., $P = 0.005$) but not in CSII alone group; however, the reduction of body weight during the 12-week visit in the two groups did not reach statistical significance (−1.6 ± 3.5 kg versus −3.3 ± 4.1 kg, for CSII alone group and CSII + Lira group, resp., $P = 0.207$) (Figure 3(d)).

5. Safety Issues

During short-term CSII therapy phase, the incidence of hypoglycemia which was defined as capillary blood glucose level <3.9 mmol/L was similar in CSII alone group and CSII + Lira group (4 (5) versus 2 (3) times per patient, $P = 0.120$). Most of the hypoglycemic episodes were mild and could be corrected after ingestion of carbohydrate. No severe hypoglycemia was recorded in either group. Gastrointestinal symptoms happened in 35% of patients in CSII + Lira group in the first few days of liraglutide injections, and most of these symptoms were well tolerated. No hypoglycemic events were reported after CSII suspension.

6. Discussion

Intensive insulin treatment was introduced in the management of newly diagnosed T2DM since 1997 [14]. By fast correction of glucotoxicity and lipotoxicity, intensive insulin

treatment is able to induce long-term glycemic remission and thereby be suggested by the latest Chinese guideline for T2DM [3, 4, 15]. In this study, a GLP-1 analog, liraglutide, was used as an add-on therapy of CSII and lasted for additional 12 weeks. As expected, liraglutide facilitated the achievement of euglycemia by shortening the time required for insulin dose titration before reaching glycemic targets. Liraglutide was also reported to reduce the daily insulin requirement in patients with more advanced T2DM treated with insulin [16]. Although the total daily insulin dosage throughout CSII treatment did not significantly differ between the two treatment groups, liraglutide significantly decreased the proportion of daily premeal bolus. Furthermore, there was also a tendency of better average glycemic control in CSII + Lira group during the 12-week extended therapy phase, as indicated by a lower HbA1c level than that in CSII alone group at the end of the follow-up. These findings were probably attributed to a better amelioration of beta cell function in CSII + Lira group compared with that in CSII alone group. These data were in accordance with previous reports on liraglutide, which showed that it reduced hyperglycemia, especially postprandial glycemic fluctuation, by glucose-dependent insulinotropic effect [17].

However, to our surprise, shortly after the suspension of liraglutide, its effect on beta cell function rapidly faded with 1 week, leading to an elevation of fasting blood glucose. The underlying mechanism for the worsening of clinical parameters remains unknown. Recently Retnakaran et al. reported that 48 weeks of liraglutide administration in patients with mean diabetes duration of 2-3 years after 4 weeks of insulin therapy also robustly increased beta cell function measured by ISSI-2 [18]. Similar to this study, they also found a rapid deterioration of beta cell function shortly after cessation of liraglutide. However, an earlier observation showed that, in patients whose blood glucose was insufficiently controlled by metformin, a prolonged treatment with exenatide for 3 years had a slight but statistical significant benefit in beta cell function 4 weeks after stopping the medicine, which was not seen in the 1-year follow-up. There are several possible explanations for the discrepancy between short-term and long-term GLP-1 analogs therapies. Firstly, because of the beneficial effects of GLP-1 analogs on beta cell proliferation and apoptosis from rodent models, liraglutide was expected to further improve functional beta cell mass [9, 10]. However, the renewal rate of beta cells in human islets was so slow that a prolonged therapy targeted at pancreatic beta cells might be necessary for an overt change in islet architecture. Less than 1 year, according to the results from both Retnakaran et al. [18] and this study, was not enough. Secondly, part of the effects of GLP-1 analogs is attributed to their effect on body weight which could help to relieve insulin resistance and restore beta cell function. In LEAD-3 monostudy, the maximum weight loss in 1.8 mg liraglutide treatment group existed in 20 weeks [11]. Although ongoing weight loss was observed in CSII + Lira group rather than CSII alone group in this study, the difference of weight loss between the two groups was not statistically significant in a relatively short treatment period (12 weeks) in a lower dose (1.2 mg/d). Thirdly, it has been well documented that GLP-1 analogs could suppress inappropriate secretion of glucagon from alpha cells. However, despite certain controversy, there are some reports showing that incretin therapy may induce hyperplasia of pancreatic alpha cells in human and rodent models [19–21]. The importance and clinical consequence of alpha cell hyperplasia are largely unknown due to lack of data, but it is not impossible that, after stopping liraglutide, the previous suppressed glucagon secretion could rebound, resulting in relapse of hyperglycemia.

Previous observations suggested that persistent improvement in insulin sensitivity was critical for long-term maintenance of near-normoglycemia [5, 6]. Our previous studies also showed that combining CSII with insulin sensitizers, that is, metformin or rosiglitazone, increases short-term remission rate by improving both insulin sensitivity and beta cell function patients with newly diagnosed T2DM. As shown in this study, 12-week treatment with liraglutide was not sufficient to cause prominent effect on insulin resistance. Enhancement of insulin action may decrease insulin demand and subsequent beta cell overload, endoplasmic reticulum stress, or oxidative stress, leading to a longer duration of glycemic remission [22, 23]. In this point of view, insulin sensitizer, other than insulin secretagogues, should be tested as combination therapy to CSII in future studies.

There were several limitations in this study. First of all, as a pilot study, the relatively small sample size may reduce the statistical power when analyzing some clinical parameters. Second, IVGTT and homeostasis model were used to evaluate beta cell function and insulin resistance. Using clamp technique as well as physiologic challenge tests such as OGTT or mix-meal test may provide further useful information.

In conclusion, liraglutide in combination with CSII could facilitate the achievement of glycemic targets and further improve beta cell function in patients with newly diagnosed T2DM. Rapid waning of beneficial effects of liraglutide implied that a prolonged treatment period might be required to obtain a sustained favorable outcome.

Conflict of Interests

There is no conflict of interests to be declared about this paper.

Authors' Contribution

Weijian Ke and Liehua Liu contributed equally to this study. Yanbing Li designed the study and reviewed the paper. Weijian Ke and Liehua Liu contributed equally to this work, including collecting and analyzing the data and writing the paper. Juan Liu, M.D., Ailing Chen, M.N., Wanping Deng, B.S.N., Pengyuan Zhang, M.D., Xiaopei Cao, M.D., Ph.D., Zhihong Liao, M.D., Ph.D., Haipeng Xiao, M.D., Ph.D., and Jianbin Liu, M.D., collected part of the data.

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Research Article

Islet Brain 1 Protects Insulin Producing Cells against Lipotoxicity

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Chronic intake of saturated free fatty acids is associated with diabetes and may contribute to the impairment of functional beta cell mass. Mitogen activated protein kinase 8 interacting protein 1 also called islet brain 1 (IB1) is a candidate gene for diabetes that is required for beta cell survival and glucose-induced insulin secretion (GSIS). In this study we investigated whether IB1 expression is required for preserving beta cell survival and function in response to palmitate. Chronic exposure of MIN6 and isolated rat islets cells to palmitate led to reduction of the IB1 mRNA and protein content. Diminution of IB1 mRNA and protein level relied on the inducible cAMP early repressor activity and proteasome-mediated degradation, respectively. Suppression of IB1 level mimicked the harmful effects of palmitate on the beta cell survival and GSIS. Conversely, ectopic expression of IB1 counteracted the deleterious effects of palmitate on the beta cell survival and insulin secretion. These findings highlight the importance in preserving the IB1 content for protecting beta cell against lipotoxicity in diabetes.

1. Introduction

Type 2 diabetes is one of the major health challenges of the 21st century. The disease arises when beta cells produce insufficient insulin to meet the increased hormone demand, caused by insulin resistance or growth of tissues such as liver, muscle, and adipose tissues. Although genome-wide association studies revealed a genetic contribution in the etiology of the disease [1], the environmental risks factors are very likely the most prominent cause of beta cell decline in the vast majority of cases [2]. Lifestyle changes such as lack of physical activity together with excessive adiposity contribute to chronic elevation of the circulating plasma saturated free fatty acids (FFAs). Numerous studies have highlighted that chronic exposure to elevated levels of FFAs, in particular palmitate, is detrimental by promoting insulin resistance and beta cell dysfunction [3]. The beta cell failure elicited by palmitate includes a defect in their secretory capacity to respond to glucose and a loss of beta cell mass by apoptosis [4–8]. These diabetogenic effects of palmitate are in part achieved by modulating the expression and activity of pro-apoptotic and antiapoptotic proteins [3, 9–20].

The mitogen activated protein kinase 8 interacting protein 1, also named islet brain 1 (IB1) or c-Jun N Terminal Kinase- (JNK-) interacting protein 1 (JIP1), is mainly expressed in islet beta cells and is one of the key antiapoptotic factors of this cell type [21–24]. Reduction of the IB1 content in insulin producing and islets cell increases apoptosis [25–27]. A wealth of data reports the diminution of IB1 level, as a major mechanism through which inflammatory cytokines

cause beta cell apoptosis [22, 23, 25–29]. Some studies have ascribed the protective role of IB1 to the regulation of JNK pathway, although the exact mechanism of this regulation is still unclear [30, 31]. Reduction of IB1 expression may activate phosphorylation of JNK targets [30]. A mutation within the coding region of this gene has been associated with a rare and monogenic form of diabetes and induces beta cell death *in vitro* [23].

Conversely, overexpression of IB1 renders cells more resistant to apoptosis induced by cytokines [22, 23, 26, 27, 29]. Moreover, induction of IB1 is a major target of the glucagon-like peptide 1 mimetics for preventing beta cell death [26]. However, the role of IB1 in the context of lipotoxicity has not been reported thus far. In this report, we demonstrated the roles of IB1 in palmitate-induced beta cell death and function and described the regulation of IB1 by palmitate at both the transcriptional and posttranslational levels.

2. Material and Methods

2.1. Materials. Palmitate (sodium salts) was obtained from Sigma-Aldrich (St. Louis, MO). The saturated fatty acid was coupled to bovine serum albumin by 1 h agitation at 37°C and freshly prepared for each experiment [32]. This procedure yielded BSA-coupled fatty acids in a molar ratio of 5 : 1. The MG132 compound was purchased from Sigma-Aldrich (St. Louis, MO). The antibodies against IB1, mSIN3, and c/ebpβ were obtained from Santa Cruz Biotechnology (CA, USA).

2.2. Islets Preparation, Cell Culture, and Transfection. Rat islets were isolated from the pancreas of Sprague-Dawley rats (male, at body weight of 250–350 g) by ductal injection of collagenase. The purification and culture of islets were conducted as described [29]. The mouse insulin-secreting cell line MIN6 was cultured in DMEM glutamax medium (Invitrogen, Carlsbad, CA) supplemented with 15% FCS, 50 U/mL penicillin, 50 μg/mL streptomycin, and 70 μM β-mercaptoethanol [33]. The rat insulin-secreting cell line INS-1E was maintained in RPMI 1640 medium supplemented with 10% FCS, 1 mM Sodium Pyruvate, 50 μM β-mercaptoethanol, and 10 mM Hepes [26]. The plasmid encoding HA-IB1-WT and siRNA duplexes directed against IB1 (si-IB1), GFP (si-GFP), or ICER (siICER) were previously described [26, 34]. Plasmids or the siRNA duplexes were introduced using the Lipofectamine 2000 (Invitrogen AG) exactly as described [26].

2.3. Measurement of Insulin Secretion. The MIN6 cells (10^5) were plated in 24-well dishes. Two days after transfection, cells were washed twice with PBS. Thereafter, cells were preincubated in KRBH buffer (140 mM NaCl, 3.6 mM KCl, 0.5 mM NaH_2PO_4, 0.5 mM $MgSO_4$, 1.5 mM $CaCl_2$, 2 mM $NaHCO_3$, 10 mM HEPES, 0.1% bovine serum albumin, and pH 7.4) containing 2 mM glucose for 1 hour. Afterward medium was changed with KRBH buffer containing 2 mM glucose corresponding to basal state or with 20 mM glucose for an additional 45 minutes. Insulin secretion was measured by EIA (SPI-BIO) according to manufactured protocol.

2.4. Western Blotting. The cells were scrapped in the PBS and lysed by using a NP-40 lysis buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, and 1% NP-40) supplemented with antiproteases and antiphosphatases (Roche). 25–40 μg of total protein extracts was separated on 10% SDS-polyacrylamide gel and electrically blotted to nitrocellulose membrane. The proteins were detected using a buffer containing 0.1% Tween 20 and 5% milk and incubated overnight at 4°C with specific primary antibodies and were visualized with IRDye 800 or IRDye700 (Rockland) as secondary antibodies. Quantification was realized using the Odyssey Infrared Imaging System (Li-COR).

2.5. Reverse Transcription Coupled with Quantitative PCR (RT-qPCR). Total RNA was extracted using guanidium thiocyanate-phenol-chloroform RNA purification method. Reverse transcription was performed as described [34]. Real-time quantitative-PCR assays were carried out on the Bio-Rad MyiQ Real-Time PCR Detection System using iQ SyBr Green Supermix (Bio-Rad) as the amplification system with 100 nM primers and 2 μL of template (RT product) in 20 μL of PCR volume and annealing temperature of 59°C. Primers sequences were as follows: mouse *Ib1*, sense 5′-ACA AGG GCA ATG ATG TCC TC-3′ and antisense 5′-TTT ATT TCC CTT GGC CTC C-3′; mouse housekeeping ribosomal protein, large P0 (*Rplp0*), sense 5′-ACCTCCTTC-TTCCAGGCTTT-3′ and antisense 5′-CCACCTTGTCTC-CAGTCTTT-3′; mouse *Bcl2*, sense 5′-CTCCCGATTCAT-TGCAAGTT-3′ and antisense 5′-TCTACTTCCTCCGCA-ATGCT-3′.

3. Results

3.1. Reduction of Ib1 Content in MIN6 Cells by Palmitate Relied on the Transcriptional Repressor ICER and Proteasome-Mediated Degradation. A large number of reports have confirmed the adverse effects of palmitate on function and survival of isolated islets and different insulin-secreting cells including MIN6 cells [11, 13, 15, 19]. For this reason we chose to monitor the *Ib1* mRNA level in MIN6 cells and isolated rat islets that were cultured with palmitate. RT-qPCR showed reduction of *Ib1* mRNA in islet and MIN6 cells cultured with palmitate for 48 and 72 hrs (Figure 1(a)). Because palmitate modulates the activity of several transcription factors [11], we tested the hypothesis that the decreased *Ib1* mRNA levels resulted from reduced transcriptional activity of its promoter. The human proximal *IB1* promoter contains several key elements that promote expression and regulation of the gene in beta cell [35]. A 731 bp fragment of the proximal promoter has been cloned upstream of the luciferase reporter (*IB1*luc) and is highly active in insulin producing cells [24]. As previously observed, luciferase activity of the IB1-luc construct was 20–25-fold higher than the promoterless control vector in MIN6 cells (Figure 1(b)). This activity was reduced by twofold when the cells were cultured in the presence of palmitate (Figure 1(b)). The *IB1* promoter contains a cAMP response element (CRE) [26]. This element binds the inducible cAMP early repressor (ICER) [26], an antagonist of the CRE-binding protein (CREB). ICER

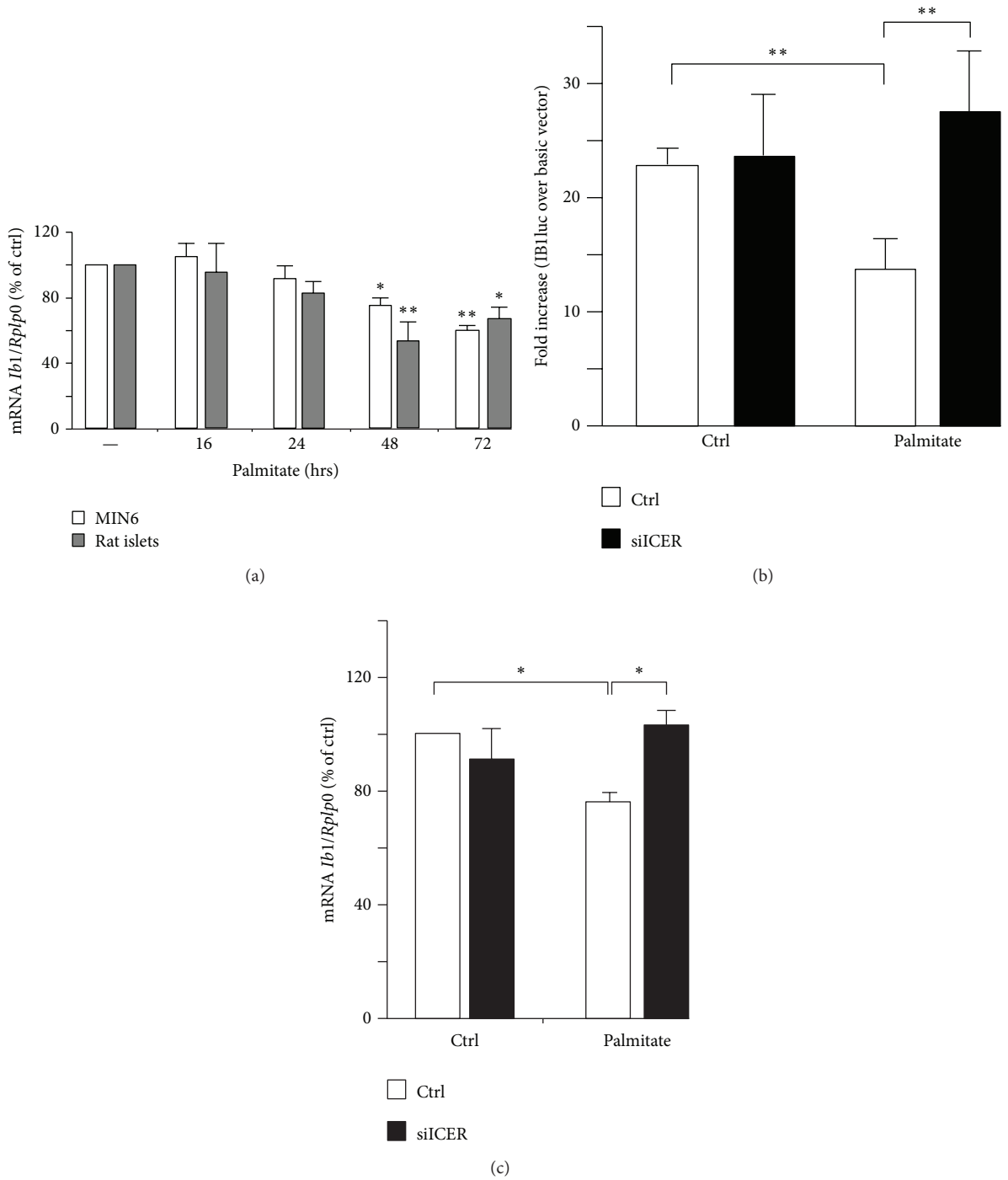

FIGURE 1: Effect of palmitate on the *Ib1* mRNA level. (a) Quantification of *Ib1* mRNA level by RT-qPCR from MIN6 cells (*open bar*) and isolated rat islets (grey bar) cultured with 0.5 mM palmitate or BSA (−) at different indicated times. (b) Assessment of Ib1 transcriptional activity in MIN6 cells cultured with palmitate. Cells were transiently transfected with a luciferase reporter construct driven by the 731 bp fragment of the human *MAPK8IP1* promoter (IB1luc). Palmitate was added to the medium 24 hrs after transfection and luciferase activity was measured 48 hrs later. To test the role of ICER, IB1luc was cotransfected together with duplexes of control small interfering RNA (siGFP, *open bar*) or siRNA directed specifically against ICER (siICER, *filled bar*). The data are expressed as fold increase over the control vector pGL3basic and are the mean ± SEM of three independent experiments. (c) Role of ICER in the drop of *Ib1* mRNA induced by palmitate. The *Ib1* mRNA was measured by RT-qPCR in MIN6 cells that were transfected with duplexes of either siGFP (*open bar*) or siICER (*filled bar*). After transfection, (24 hrs) the cells were cultured with BSA (ctrl) or 0.5 mM palmitate for additional 48 hrs. The results were normalized against *Rplp0* and the expression levels from cells cultured with BSA were set to 100%. Data are the mean ± SEM of 3 independent experiments ($^{**}P < 0.01$; $^{*}P < 0.05$).

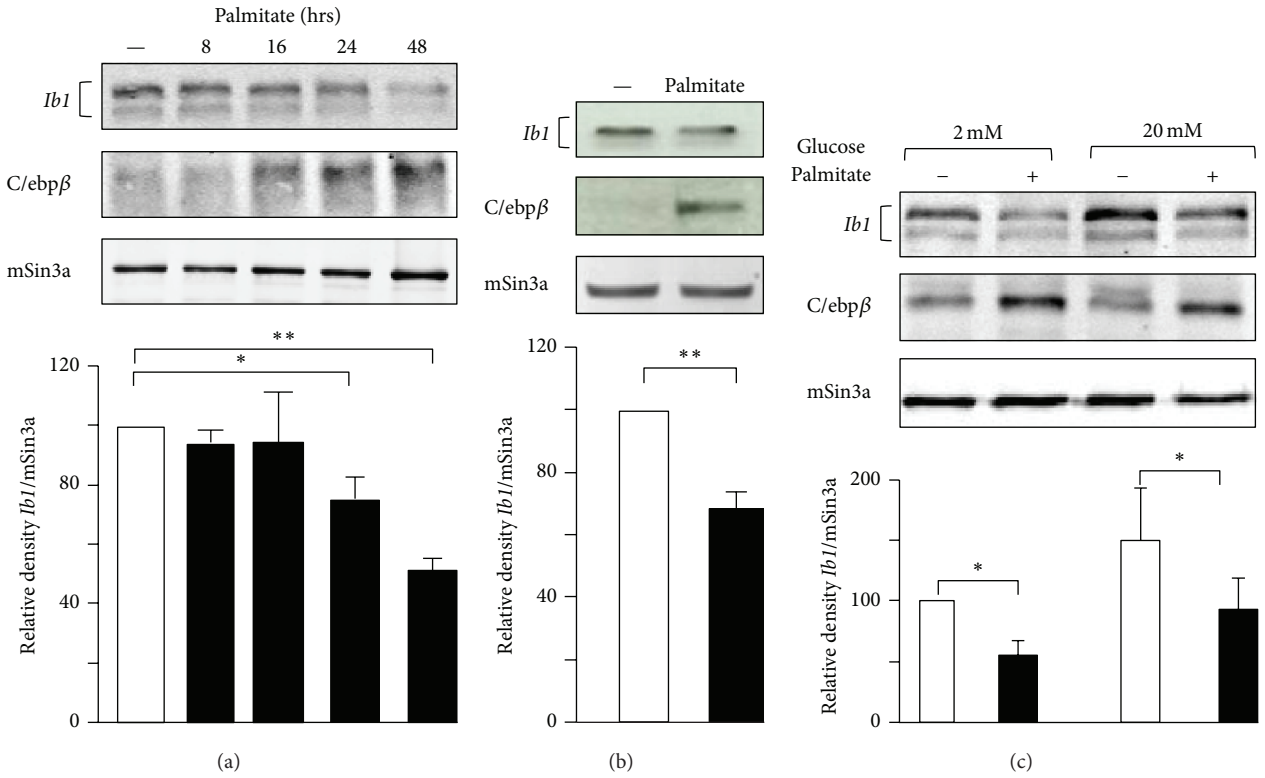

Figure 2: Effect of palmitate on the Ib1 protein level. Total protein was prepared from (a) MIN6 cells or (b) isolated rat islets that were cultured with 0.5 mM palmitate or BSA (−) at different indicated incubation times and for 24 hrs, respectively or (b) incubated with BSA or palmitate and (c) INS-1E exposed to BSA (−) or 0.5 mM palmitate in the presence of 2 mM or 20 mM glucose. As a positive control for the palmitate efficacy, the expression of C/ebpβ was monitored. Immunoblotting of mSin3a was done as negative control. The *Graphs* below the blots depict the densitometric analysis. The sum of the Ib1 band intensities of cells treated with BSA was set at 100%. The figure shows the results of a representative experiment out of five. Data are the mean of ± SEM of 3 independent experiments ($^{**}P < 0.01$; $^{*}P < 0.05$).

expression rises up in beta cells incubated with palmitate [36]. We have previously demonstrated that overexpression of ICER represses the promoter activity of *IB1*-luc in beta cells [26]. To investigate whether ICER links palmitate to reduced Ib1 mRNA levels, we transfected MIN6 cells with siRNA directed against ICER (siICER) that we previously validated in beta cells [28, 34, 37]. Interestingly, silencing of ICER restored *IB1*luc activity and *Ib1* mRNA levels in the presence of palmitate (Figures 1(b) and 1(c)), supporting a role for ICER in the reduction of *Ib1* expression induced by palmitate. Activating transcription factor 3 (ATF3), which also binds to the CRE site, is a potent repressor of gene expression induced by palmitate in beta cells [35, 38, 39]. However, the Ib1 expression was neither reduced in cells in which Atf3 was overexpressed (see supplementary Figure 1a in Supplementary Material available online at http://dx.doi.org/10.1155/2016/9158562) nor increased in islets cells from atf3 knockout mice, thus ruling out a role for Atf3 in the loss of *Ib1* mRNA caused by the saturated fatty acid (supplementary Figure 1b). Two Ib1 isoforms, one corresponding to the full length protein and one from the use of an alternative promoter [26], were detected in MIN6 cells by immunoblotting experiments (Figure 2(a)) [26, 27]. A significant reduction in Ib1 protein levels was apparent after 24 hrs treatment of cells with palmitate (Figure 2(a)). These results were confirmed in isolated rat islets cultured with palmitate for 24 hrs (Figure 2(b)). Palmitate hampers insulin expression, secretion, and cell survival by inducing the expression of C/EBPβ [11]. Interestingly we observed that the decreased Ib1 protein level was concomitant with the increased C/EBPβ protein levels (Figures 2(a) and 2(b)). Chronic hyperglycemia potentiates the harmful effects of palmitate [9] in INS-1E cells but not in MIN6 or isolated human islets [40]. To determine whether the effects of palmitate were potentiated by glucose, Ib1 protein levels were quantified in INS-1E cells cultured with palmitate in the presence of low or high glucose concentration (5 or 20 mmol/L glucose, resp.). A similar reduction of Ib1 by palmitate was observed upon low or high glucose concentration (Figure 2(c)), indicating that palmitate decreases the expression of high glucose concentration. Since decreased Ib1 protein levels occurred prior to the decrease of its mRNA levels (Figures 1(a) and 2(a)), this suggests that the reduction of *Ib1* mRNA is not the only mechanism affecting its protein content. The fatty acid affects beta cell survival and function through ER stress dependent pathways [3, 15–17]. Palmitate impairs Ca^{2+} influx to ER by affecting

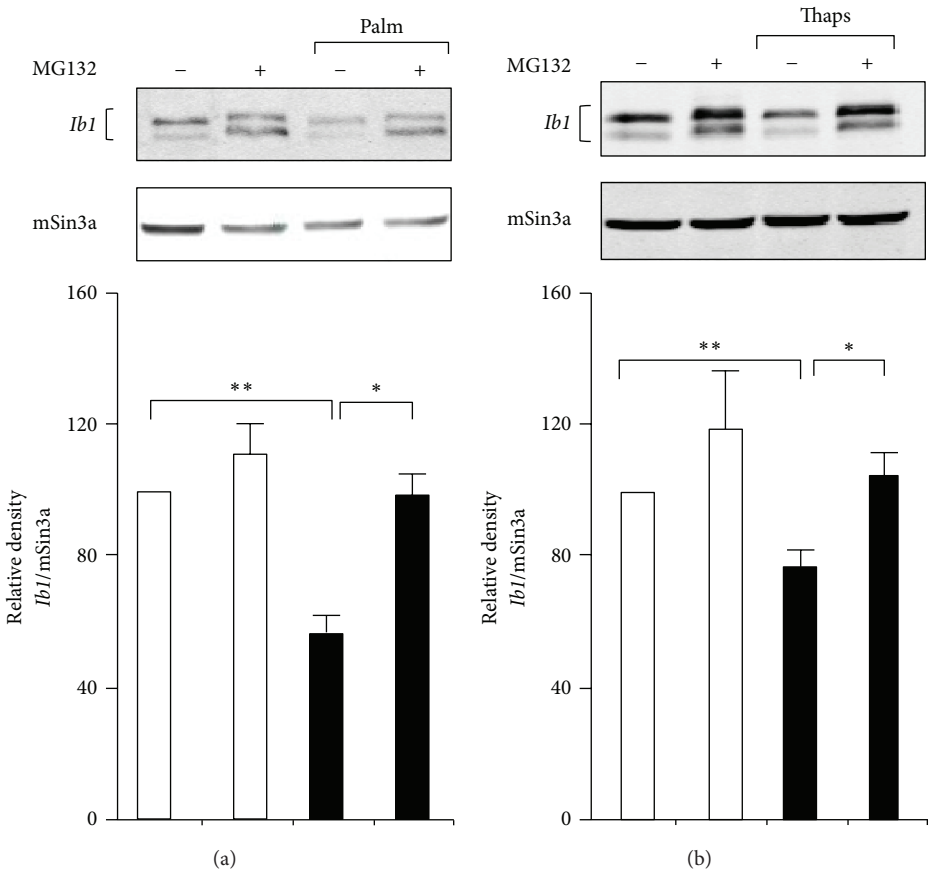

Figure 3: Effect of the proteasome inhibitor MG132 on the decrease of Ib1 content caused by palmitate. Ib1 content was measured from MIN6 cells exposed to (a) 0.5 mM palmitate or (b) 1 μM thapsigargin (thaps) for 48 hrs in the presence or absence of 1 μM of the proteasome inhibitor MG132. The Ib1 level was normalized against the mSin3a. The figure shows the result of a representative experiment out of three. The results are expressed as the mean ± SEM of three independent experiments ($^{**}P < 0.01$; $^{*}P < 0.05$).

sarcoendoplasmic-reticulum pump Ca^{2+}-ATPase (SERCA), also known as ATP2A2 activity [19]. Defective cytosolic Ca^{2+} leads to proteasome-mediated degradation [41]. To test the hypothesis that the loss of Ib1 content involves proteasome, MIN6 cells were coincubated with palmitate and the proteasome inhibitor MG132. Treatment of cells with this chemical compound efficiently restored Ib1 protein levels in the presence of palmitate (Figure 3(a)). In addition, thapsigargin (thaps), an ER stress inducer that promotes Ca^{2+}-induced degradation evoked by proteasome [42], reduced Ib1 protein content in MIN6 and INS-1E cells (Figure 3(b)).Under these experimental conditions, MG132 treatment efficiently restored Ib1 protein level in the presence of thaps (Figure 3(b)).

3.2. Overexpression of Ib1 Counteracts the Deleterious Effects of Palmitate on Glucose-Induced Insulin Secretion and Cell Survival. IB1 is required for glucose-induced insulin secretion and cell survival [23, 26, 43]. We investigated whether the decreased Ib1 level contributes to palmitate-induced cell death by ectopically expressing Ib1. As shown in Figures 4(a) and 4(b), IB1 partially rescued the cells as evidenced by the reduction in apoptotic cell number and increase in the mRNA level of *Bcl2*, an antiapoptotic gene. Conversely, silencing of Ib1 using a previously validated siRNA [26] potentiated the effect of palmitate on cell death (Figure 4(a)) with a concomitant increase of the *Bcl2* mRNA (Figure 4(b)). We next investigated whether the reduction of Ib1 by palmitate could contribute to defective glucose-induced insulin secretion. As previously shown [43], silencing of Ib1 in Min6 cells reduced glucose-induced insulin secretion (Figure 5), which was exacerbated in the presence of palmitate (Figure 5). Transient ectopic expression of Ib1 can overcome proteasome-mediated degradation of Ib1 elicited by cytokines [22, 23, 27, 44]. It has been previously shown that Ib1 overexpressing beta cells are more resistant to apoptosis [22, 23, 27, 44]. Interestingly and in line with these observations, Ib1 overexpressing Min6 cells improved their glucose-induced insulin secretion when chronically exposed to palmitate (Figure 5).

4. Discussion

Evidence for the potential diabetogenic role of palmitate by afflicting beta cell function and survival has been provided

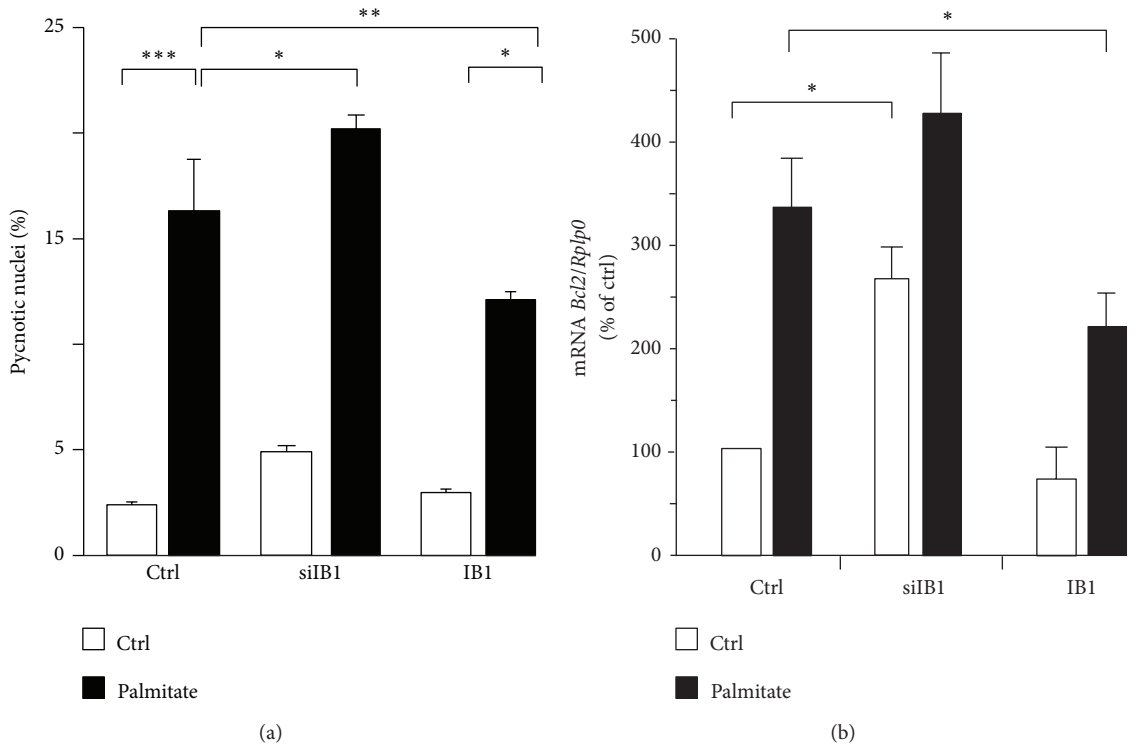

Figure 4: Role of Ib1 in apoptosis induced by palmitate. (a) MIN6 cells were transfected with the siRNA duplex directed against IB1 (siIB1) or control siRNA (siGFP, Ctrl) or the plasmids coding for the wild type HA-tagged IB1 (IB1). For scoring death, 0.5 mM palmitate (*filled bars*) or BSA (Ctrl, *open bars*) was added to the cells medium 24 hrs after transfection. The rate of apoptosis was scored by counting pycnotic nuclei in cells exposed for 48 hrs to palmitate. Results are expressed as mean ± SEM of 3 independent experiments ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). (b) For the quantification of *Bcl2*, total RNA from transfected cells with siGFP, siIB1, and IB1 was prepared and subjected to qPCR. The levels of *Bcl2* were compared in cells incubated with BSA (*open bars*) and 0.5 mM palmitate (*filled bars*) for 48 hrs. The mRNA were normalised against *Rplp0* and those of the control cells were set to 100%. Data are the mean ± SEM of five independent experiments ($^{*}P < 0.05$).

by a plethora of data from *in vitro* and *in vivo* experiments [3, 7, 12, 41]. Palmitate decreases beta cell survival by promoting apoptosis [19]. Reduction of the antiapoptotic IB1 expression is a major mechanism eliciting beta cell apoptosis in response to cytokines and oxidized LDL [25, 26, 28, 29]. However, its role in lipotoxicity has not been reported. In this report, we show that palmitate decreases Ib1 gene expression at both the transcriptional and posttranslational levels. At the transcriptional level, the effect is dependent on the transcriptional repressor ICER, since silencing of ICER dampened the ability of palmitate to reduce *Ib1* mRNA. Although the level of ATF3, another transcriptional repressor, is increased by palmitate [39], our data indicate that ATF3 is not necessary for palmitate to repress Ib1 gene expression. Interestingly, our data showed that diminution of IB1 protein content occurs earlier than the drop of *Ib1* mRNA and this was via a proteasome-mediated pathway. Overexpression of Ib1 protects beta cell against apoptosis triggered by cytokines [22, 25]. In line with this protective effect we observed that ectopic expression of Ib1 prevented the deleterious effect of palmitate on cell survival.

Beside its antiapoptotic role, Ib1 regulates glucose-induced insulin secretion [21, 23, 43]. Consistent with this metabolic function, inactivation of Ib1 alters insulin secretion stimulated by glucose [43]. Herein we confirmed that silencing of Ib1 mimics the effect of palmitate on insulin secretion. Moreover, ectopic expression of Ib1 partially restored glucose-induced insulin secretion in response to palmitate, indicating that exogenous expression of Ib1 compensates for the decrease of Ib1 content caused by palmitate. Ib1 is described as a scaffold protein that assembles the kinases involved in the JNK activation; however, paradoxically its function is to inhibit JNK activity [22, 25, 26, 29, 31]. JNK activation often (but not always) precedes JNK activity. The c-Jun transcription factor is a JNK target that is deemed to couple JNK activation to apoptosis [45]. JNK phosphorylates c-Jun and this could lead to apoptosis [45, 46]. Independent studies have shown that Ib1 level may be required for inhibiting phosphorylation of c-Jun [25, 27]. Overexpression of Ib1 level reduces phosphorylation of c-Jun caused by cytokines in islets and insulin producing cells [25, 27].

JNK pathway is activated in response to several diabetogenic stresses including oxidized LDL and cytokines [26, 29]. Increased JNK activity is a key mechanism coupling palmitate to beta cell dysfunction and ultimately cell death [3, 19], and inhibition of JNK activity alleviates the adverse

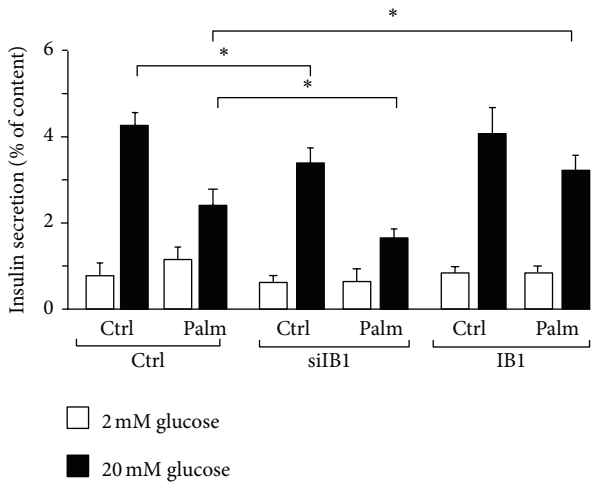

Figure 5: Role of Ib1 in impaired glucose-induced insulin secretion caused by palmitate. For measurement of insulin secretion, MIN6 cells were transiently transfected with the control siRNA (*open bar*) or siIB1 (*filled bar*) or the plasmids coding for the wild type HA-tagged IB1 (IB1). 48 hrs after transfection, insulin secretion was stimulated by preincubating the cells for 30 min in Krebs-Ringer buffer containing 2 mmol/L glucose and, thereafter, incubating the cells with glucose 20 mmol/L. The amounts of insulin release and cellular contents during the incubation period were measured by EIA. The results are expressed as the ratio between the amounts of insulin released over the content and are the mean ± SEM of three independent experiments.

effects of palmitate [3, 19]. Thus, our finding that palmitate reduces Ib1 expression may provide a potential mechanism for palmitate to increase JNK activity. There are three JNK isoforms identified so far [47]. All of them are present in beta cells [48]. There are growing studies pointing to divergent roles in JNK isoforms in beta cells. While JNK2 seems to be proapoptotic, JNK1 and JNK3 are antiapoptotic [48–50]. Therefore, further analyses are required to determine whether and how Ib1 may regulate each of the JNK isoforms. Understanding such regulation will permit us to elucidate the mechanism through which IB1 preserves beta cell against the harmful effects caused by palmitate. Inhibition of the JNK pathway has been proposed as a potential therapeutic way for treating beta cell failure in type 2 diabetes and some efforts are currently maintained to identify novel JNK inhibitors [51]. Future investigation of IB1 activity may help in finding out novel targets exploitable in the design of next innovative therapies of T2D.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Saška Brajkovic, Mourad Ferdaoussi, and Valérie Pawlowski equally contributed to the work.

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Review Article

Roles of Pyruvate, NADH, and Mitochondrial Complex I in Redox Balance and Imbalance in β Cell Function and Dysfunction

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Pancreatic β cells not only use glucose as an energy source, but also sense blood glucose levels for insulin secretion. While pyruvate and NADH metabolic pathways are known to be involved in regulating insulin secretion in response to glucose stimulation, the roles of many other components along the metabolic pathways remain poorly understood. Such is the case for mitochondrial complex I (NADH/ubiquinone oxidoreductase). It is known that normal complex I function is absolutely required for episodic insulin secretion after a meal, but the role of complex I in β cells in the diabetic pancreas remains to be investigated. In this paper, we review the roles of pyruvate, NADH, and complex I in insulin secretion and hypothesize that complex I plays a crucial role in the pathogenesis of β cell dysfunction in the diabetic pancreas. This hypothesis is based on the establishment that chronic hyperglycemia overloads complex I with NADH leading to enhanced complex I production of reactive oxygen species. As nearly all metabolic pathways are impaired in diabetes, understanding how complex I in the β cells copes with elevated levels of NADH in the diabetic pancreas may provide potential therapeutic strategies for diabetes.

1. Introduction

Complex I (NADH-ubiquinone oxidoreductase) is the primary electron entry point in mitochondrial electron transport chain [1, 2] and is absolutely required for glucose-stimulated insulin secretion [3]. In mammalian cells, complex I has at least 45 subunits with a molecular weight close to 1000 kDa [4–6]. This huge complex has three major functions in mitochondrial bioenergetics and oxygen consumption (Figure 1). First, it is the major enzyme that oxidizes NADH to NAD^+; thus, it is responsible for regenerating the majority of NAD^+ for continued glycolysis and for the function of NAD^+-dependent enzymes such as sirtuins, CD38, and poly ADP ribose polymerases (PARPs) [7–11]. Second, complex I is the major proton pumping machine in the mitochondrial inner membrane [2, 12], which drives mitochondrial ATP production needed by nearly all cells. Third, complex I is the major site for cellular production of reactive oxygen species (ROS) [13, 14] that have been demonstrated to be involved in cell survival and death mechanisms [15, 16]. Interestingly, despite numerous studies on complex I and its implications in a variety of diseases [17–22], the role of complex I in β cells in the diabetic pancreas remains unknown, albeit normal function of complex I [3] and a basal level of complex I-generated ROS are needed for insulin secretion under physiological conditions [23]. In this review, we discuss the fate of glucose, mechanisms of insulin secretion, and the roles of glucose metabolic pathways including pyruvate cycling and NADH cycling in insulin secretion under physiological conditions. We propose the hypothesis that complex I is a key player in maintaining redox balance for β cell insulin secretion and that its dysfunction impairs β cell function.

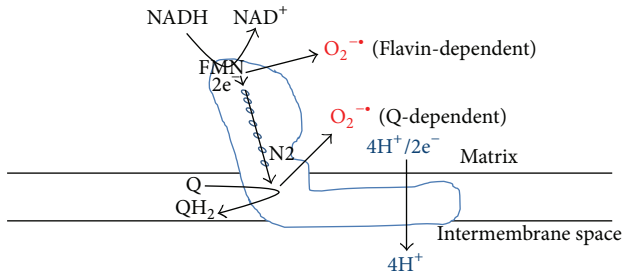

Figure 1: The three key roles of mitochondrial complex I: NADH oxidation and recycling, superoxide production, and proton pumping. Electrons from NADH are transported to CoQ via seven Fe-S clusters with the terminal one being N2 [6]. Superoxide could be produced at both the FMN-dependent site and the CoQ-dependent site [132].

2. Fate of Glucose under Physiological Conditions

Glucose can be metabolized to form numerous biomolecules [24, 25] (Figure 2). It is used for ATP synthesis via the glycolytic and oxidative phosphorylation pathways. It generates the reducing equivalent NADPH for anabolism and ribose 5-phosphate for nucleotide synthesis via the pentose phosphate pathway [26]. It can be converted via pyruvate to alanine and can also be converted to lactate when the oxygen supply is limited [24]. Furthermore, ketone bodies, sterols, and fatty acids can all be synthesized from glucose via pyruvate-derived acetyl-CoA [24].

3. β Cell Glucose Sensing and Insulin Secretion

In β cells, glucose not only is a fuel, but also stimulates insulin secretion [27–30]. Because β cells have a high-Km glucose transporter 2 (Glut2) and high-Km glucokinase, they can respond to elevated levels of blood glucose, which is known as supply-driven metabolism [31, 32]. Therefore, there is a tight link between glucose metabolism and insulin secretion [33–35]. The canonical events that trigger insulin secretion after a meal are outlined in Figure 3 [35–38]. Glucose is transported into β cells by the glucose transporter 2 (Glut2). Once inside the cells, glucose is phosphorylated by glucokinase to yield glucose-6-phosphate (G-6-P) [39, 40], which is then converted to 2 molecules of pyruvate by the glycolytic pathway. Pyruvate is then transported into mitochondria and converted to acetyl-CoA by the pyruvate-dehydrogenase complex. Acetyl-CoA then enters the tricarboxylic acid (TCA) cycle and electrons derived from it are donated to NAD^+ and FAD, leading to generation of intramitochondrial NADH and $FADH_2$. Electrons stored in these two molecules are further donated to coenzyme Q (CoQ) via complex I and complex II, respectively. The eventual electron transportation to O_2 leads to a proton gradient formation across the inner mitochondrial membranes, which drives ATP synthesis via complex V. When blood glucose levels are elevated, more NADH and ATP are produced, leading to closure of ATP-sensitive potassium channels, which in turn depolarizes cell membranes and consequently opens voltage-gated Ca^{2+} channels, resulting in Ca^{2+} influx into the cells [30]. It is this Ca^{2+} influx that triggers the initial phase of insulin secretion from prestored insulin granules after nutrient ingestion (Figure 3) [28, 35, 41].

Once stored insulin is depleted, a second phase of insulin release is initiated [42, 43]. This phase of insulin release is K_{ATP} channel-independent [29] and this phase is prolonged as insulin has to be synthesized, processed, and released for the length of time of elevated blood glucose. This phase also regenerates stores of insulin depleted in the first phase of insulin secretion and is likely stimulated by metabolites such as NADPH and α-ketoglutarate produced by pyruvate cycling pathways involving TCA cycle intermediates such as citrate, malate, and oxaloacetate [29, 37, 44].

4. Pyruvate Cycling, Conversion of NADH to NADPH, and Insulin Secretion

As an intermediate of glucose metabolism in β cells, pyruvate plays an important role in redox cycling between NADH and NADPH [41, 45, 46]. This is reflected by the three pyruvate cycling pathways across the mitochondrial membranes (Figure 4). The first is pyruvate-malate pathway. In this pathway, pyruvate is converted to oxaloacetate by pyruvate carboxylase. The latter is converted to malate by mitochondrial malate dehydrogenase. Malate is then shuttled out of mitochondria to the cytosol whereby it is converted back to pyruvate. This process results in the net formation of NADPH from NADH. The second pathway is the pyruvate-citrate pathway, in which citrate is transported out of mitochondria into the cytosol whereby it is split by citrate lyase to yield acetyl-CoA and oxaloacetate. Acetyl-CoA can be used as the carbon source for fatty acid synthesis and oxaloacetate can be converted by malic enzyme 1 to pyruvate that then reenters mitochondria. Similar to the pyruvate-malate pathway, the pyruvate-citrate pathway also results in the net formation of NADPH from NADH. The third pathway is pyruvate-isocitrate pathway involving cytosolic isocitrate dehydrogenase that uses NADP as its cofactor [47]. Therefore, reducing equivalents again are transferred from NADH to NADPH.

Evidence supporting the role of the three pyruvate cycling pathways and NADPH in insulin secretion comes mainly from the following studies. (A) Both pharmacological inhibitors and siRNA-mediated suppression of mitochondrial pyruvate carrier severely impair insulin secretion [48]. (B) siRNA-mediated suppression of malic enzyme 1 impairs insulin secretion [49]. (C) β cells have high levels of pyruvate carboxylase activity [44, 50]. Unlike liver and kidney cells that have phosphoenolpyruvate carboxykinase (PEPCK) used for gluconeogenesis, β cells do not have detectable PEPCK [44, 51]. Therefore, β cell pyruvate carboxylase must have a purpose other than gluconeogenesis, which is thought to replenish oxaloacetate in the TCA cycle when oxaloacetate is removed for the pyruvate-malate pathway to generate NADPH [52]. It has been reported that in β cells nearly

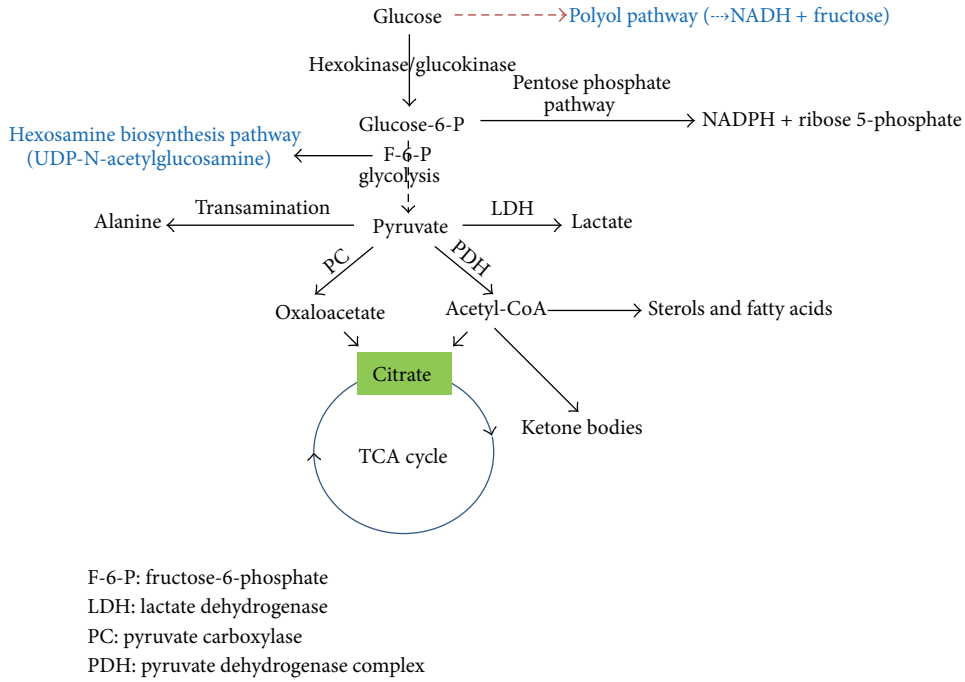

Figure 2: Fate of glucose. The major pathways shown in this diagram are glycolysis, the TCA cycle, and the pentose phosphate pathway. Additionally, glucose can be used as sources for fatty acid synthesis and lactate and alanine formation and can also be stored as glycogen in liver and skeletal muscle (not shown in the diagram). The pathways in blue (the polyol pathway and the hexosamine pathway) can be significant ones for glucose utilization under diabetic conditions.

50% of the pyruvate pool derived from glucose is converted to oxaloacetate [53]. Oxaloacetate not only replenishes the TCA cycle intermediates, but also drives the pyruvate-malate cycling pathway for NADPH production. Therefore, pyruvate and NADPH are thought to be essential triggers for β cell insulin secretion [54–57].

5. Production and Recycling of NADH

5.1. NADH Production Pathways. Electrons derived from glucose metabolism are stored in NADH and $FADH_2$, respectively. In terms of glucose combustion, NADH is mainly generated by the glycolytic pathway, by pyruvate dehydrogenase complex via dihydrolipoamide dehydrogenase [58], and by the TCA cycle [59]. As shown in Figure 5, degradation of one molecule of glucose can yield 8 molecules of NADH and two molecules of $FADH_2$ (note that one molecule of glucose drives two cycles of the TCA cycle).

5.2. Redox Shuttles for NADH Transportation. For β cells, NADH produced during glycolysis is required for glucose sensing [60] and has to be transported into mitochondria for oxidation by complex I. This is because, while in most tissues lactate dehydrogenase can regenerate NAD^+ for glycolysis to continue, β cells have very low lactate dehydrogenase activity [61]. The translocation of NADH from cytoplasm to mitochondria is achieved by two redox shuttles (Figure 6): the malate-aspartate shuttle and the glycerol phosphate shuttle [62–64]. While the malate-aspartate shuttle can directly feed NADH to complex I, the glycerol phosphate shuttle only transports electrons from NADH to $FADH_2$ that donates its electrons to CoQ via complex II. Therefore, the glycerol phosphate shuttle is less efficient in terms of energy production [24]. Another difference between the two redox shuttles is that the malate-aspartate shuttle is a reversible process that can only be activated by high level cytosolic NADH, while the glycerol phosphate shuttle is an irreversible process that can transport NADH into mitochondria even when the cytosolic NADH level is low [24]. The two redox shuttles have been shown to be important for glucose-induced insulin secretion [63–65] as blocking of both shuttles abolished glucose-induced insulin secretion although deficiency of either shuttle singly did not alter the response to glucose stimulation [28].

5.3. Complex I and NADH Recycling. Under aerobic oxidation, nearly all NADH molecules generated by glycolysis in β cells will need to be recycled by complex I (Figure 1) so that further glucose degradation can continue. It is known that even under resting conditions the rates of NADH and pyruvate generation are faster than the rates they are used [66]. Therefore, we propose that complex I is the major enzyme maintaining NAD^+/NADH redox balance and should be under constant electron pressure. While complex I's oxidation of NADH is the major pathway for NADH/NAD^+ recycling, part of NADH can also be used for reducing equivalent transfer to NADPH via the pyruvate cycling pathways as shown in Figure 4. However, whether there is

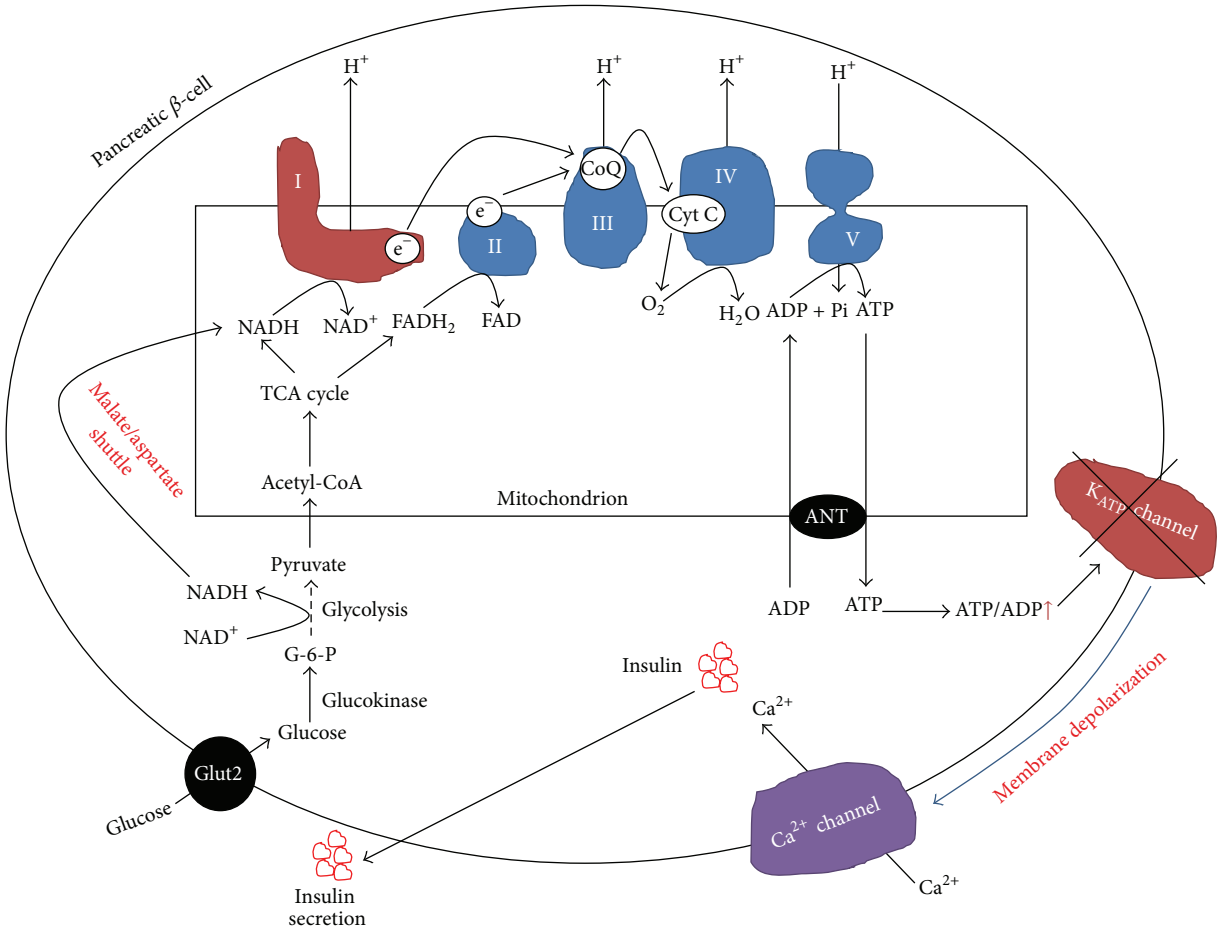

Figure 3: Mechanisms of β cell glucose sensing and insulin secretion. Shown is the first phase of insulin secretion stimulated by glucose derived ATP. When glucose levels are high, ATP levels are high, which depolarizes cell membranes, triggers the closure of the K_{ATP} channels, and induces opening of the Ca^{2+} channel. Consequently, insulin granules are infused with membranes and insulin is released. Complex I plays a key role in this process as ATP production is driven by its oxidation of NADH and transportation of electrons to CoQ that accompany proton pumping needed for ATP synthesis by complex V.

any crosstalk between complex I and the pyruvate cycling pathways remains unknown at the present time.

6. Fate of Glucose in Diabetes

Under diabetic conditions, the glycolytic pathway is usually impaired, not only due to inhibition by elevated levels of NADH resulting from overnutrition or fuel excess [25, 67], but also due to impairment of glycerol-3-phosphate dehydrogenase that is very vulnerable to oxidative and post-translational modifications [68–71]. The consequence of this impairment is that the flux of glucose through otherwise insignificant glucose metabolic pathways is increased. These include the polyol pathway and the hexosamine pathway (Figure 2, the pathways in blue), PKC activation, and the advanced glycation pathway [72]. Each of these pathways has been demonstrated to be involved in ROS production and induction of oxidative stress [71]. Therefore, oxidative stress has been postulated to be a unifying mechanism by which diabetes and its complications develop [73, 74].

7. The Polyol Pathway and NADH/NAD^+ Redox Imbalance

Since the polyol pathway generates NADH that can be fed into complex I via the malate-aspartate shuttle, we would like to discuss the role of this pathway in diabetes in a little more detail. The pathway involves two steps (Figure 7(a)). The first reaction is glucose reduction by aldose reductase to form sorbitol. This step consumes NADPH, so $NADP^+$ is formed. In certain tissues, sorbitol can accumulate and impair cellular function by altering osmolarity [75, 76]. The second reaction is sorbitol oxidation by sorbitol dehydrogenase to form fructose. This reaction uses NAD^+ as the oxidant and generates NADH and has been thought to be a major contributing factor to NADH/NAD^+ redox imbalance and pseudohypoxia as it can compete with GAPDH for NAD^+ [77], thereby decreasing cytosolic level of NAD^+ [78–80]. Intriguingly, the rates of both glycolysis and the polyol pathway are known to be increased in diabetic hyperglycemia [76], but how complex I handles the additional amount of NADH produced by

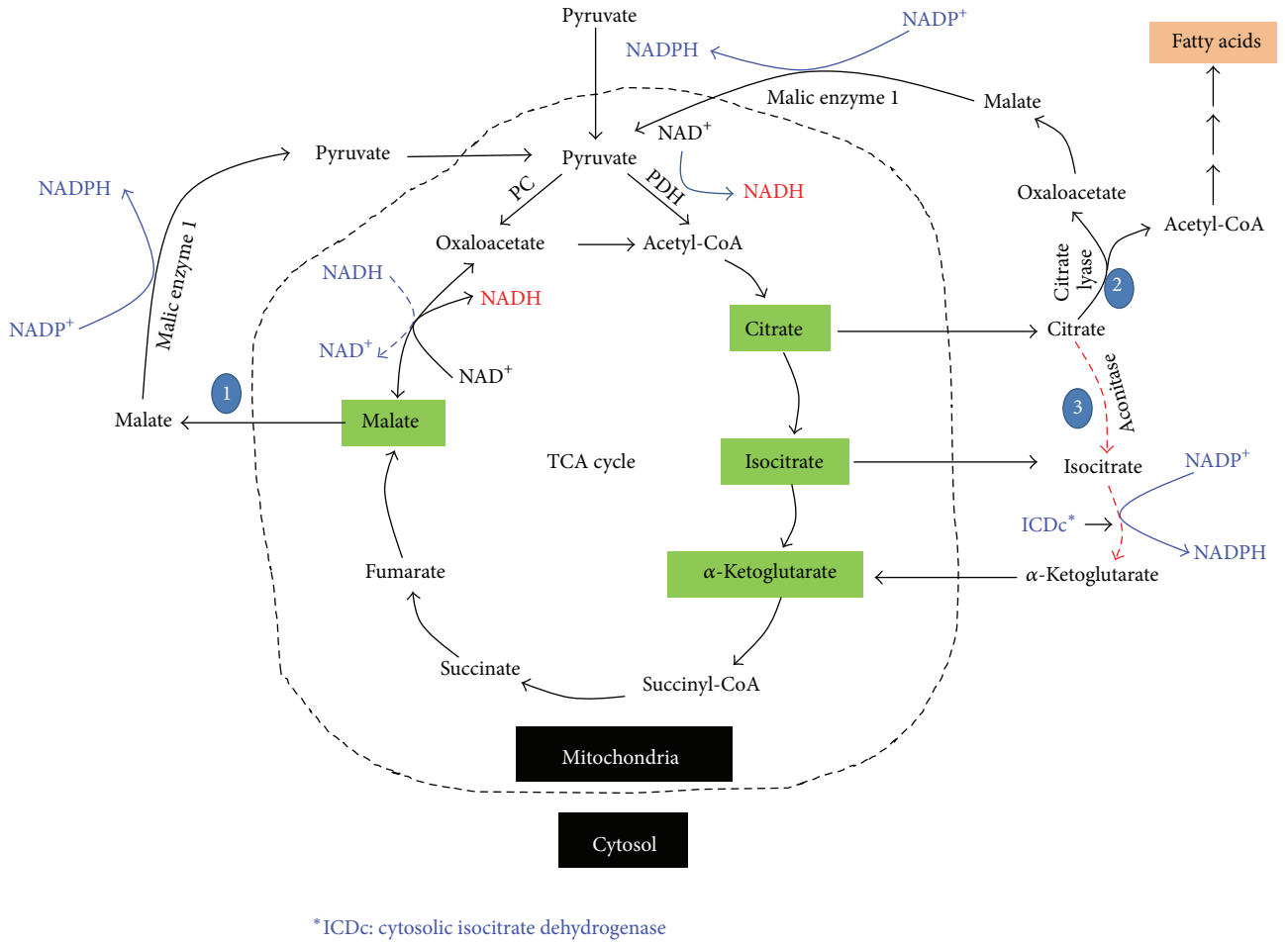

Figure 4: The pyruvate recycling pathways in β cell mitochondria. The three pathways shown are pyruvate-malate pathway, the pyruvate-citrate pathway, and the pyruvate-isocitrate pathway. Each pathway converts reducing equivalents from NADH to NADPH that plays key roles in the second phase of insulin secretion.

the polyol pathway is unknown. Moreover, it should be noted that the accumulation of fructose has been suggested to be more deleterious than that of glucose [75, 81] as fructose metabolism by fructokinase bypasses key-regulated steps of the glycolytic pathway [82] and thus can deplete intracellular phosphate and ATP, thereby inducing oxidative stress and inflammation [83].

Interestingly, as the first reaction consumes NADPH, it has been suggested that consumption of NADPH by the polyol pathway can also contribute to oxidative stress because a lower level of NADPH would impair glutathione synthesis by NADPH-dependent glutathione reductase. However, conclusive evidence that NADPH levels or alterations in NADPH/NADP$^+$ ratios are lower in diabetes has yet to be established. In fact, it has been reported that NADPH levels in certain diabetic tissues are higher [75, 76, 84], though the underlying mechanisms remain unknown. It is likely that the pyruvate cycling pathways could generate the majority of NADPH in diabetes.

8. Complex I and β Cell Dysfunction in the Diabetic Pancreas

During diabetes, many metabolic pathways are impaired due to persistent hyperglycemia. At the early stages of hyperglycemia, elevated levels of NADH are mainly produced by the conventional glucose metabolic pathways including glycolysis and the TCA cycle. As more NADH is produced, more electron pressure would be imposed on complex I. In this sense, complex I dysfunction would likely mean increased complex I activities as more NADH needs to be handled by complex I. Indeed, it has been reported that complex I activity is elevated in streptozotocin-induced diabetic rats [85, 86]. Furthermore, as NADH oxidation by complex I is accompanied by electron flow associated with electron leakage and partial oxygen reduction [87, 88], more NADH oxidation would thus lead to more ROS production [89]. This would eventually impair the glycolytic pathway due to inhibition of glycerol-3-phosphate dehydrogenase by reduced

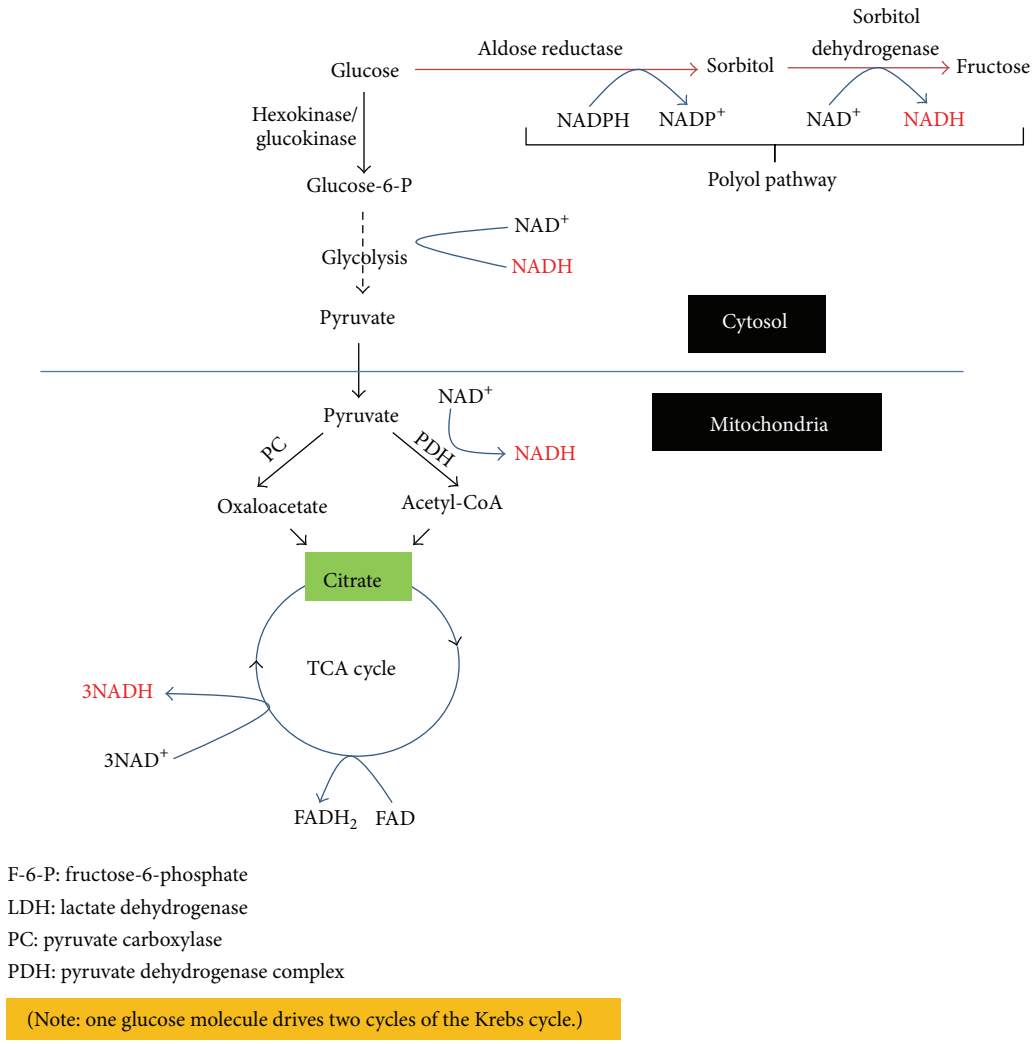

FIGURE 5: Pathways of NADH production during glucose combustion. These are the glycolytic pathway, the pyruvate dehydrogenase complex, and the TCA cycle. Under diabetic conditions, the polyol pathway also becomes a significant pathway for NADH production that can further perturb the redox balance between NADH and NAD^+.

availability of NAD^+ [68, 69, 71, 90–94], leading to diversion of glucose to other disposal pathways such as the polyol pathway [95, 96]. It has been estimated that under diabetes approximately 30% of the glucose is metabolized by the polyol pathway [93]. As this pathway generates NADH from NAD^+, the ratio of NADH to NAD^+ is highly elevated and perturbed [73, 79, 97], leading to enhanced ROS production [98, 99] and establishment of a chronic pseudohypoxic condition that can cause chronic inflammation known to be contributing to β cell dysfunction [100–102]. Hence, there is a problem in NADH and NAD^+ recycling in diabetes, suggesting that complex I function is impaired. We incline that complex I activity would be elevated in diabetic pancreas as more NADH has to be recycled by complex I. Nonetheless, how complex I function is indeed impaired (either an increase or a decrease in activity) by diabetic hyperglycemia in β cells has yet to be investigated. It is our belief that, under diabetic conditions, a smooth flow of NADH via complex I could help fight diabetes. On one hand, NADH is overproduced due to overnutrition and hyperglycemic activation of the polyol pathway [81, 103]. On the other hand, the NAD^+ level is getting lower and possibly facing depletion due to potential impairment in complex I activity and activation of NAD^+-dependent enzymes such as sirtuins, CD38, and poly ADP ribose polymerase [10, 104–106]. Indeed, it has been established that overactivation of the NAD^+-dependent PARP can trigger cell death due to NAD^+ depletion [69, 107, 108]. Therefore, an efficient NADH oxidation by complex I in diabetes would be beneficial for diabetic individuals.

Based on the above discussions, we postulate that complex I represents a potential therapeutic target for diabetes. Specifically, as proposed in a hypothetical model shown in Figure 7(b), if a protein or a small molecule target could be designed under diabetic conditions to reduce metabolic pressure on complex I, that is, relaying excess electrons from NADH to CoQ, such a target could serve as a potential therapeutic approach by restoring NADH/NAD^+ redox balance in the absence of enhanced proton pumping and

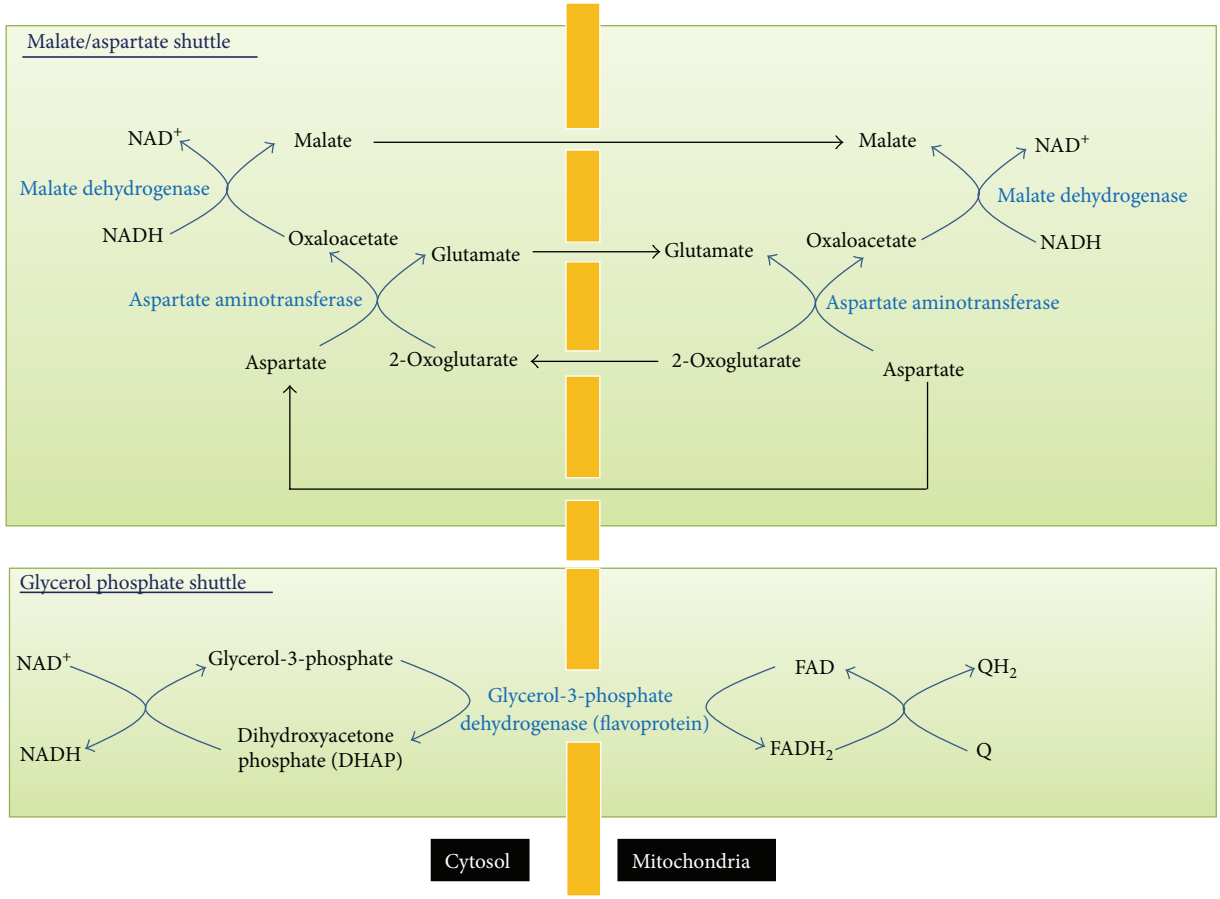

Figure 6: The two redox shuttles transporting cytosolic NADH into mitochondria. These are malate/aspartate shuttle and the glycerol phosphate shuttle. The former is reversible and only transports NADH when cytosolic NADH levels are high; the latter is irreversible and can transport NADH from cytosol to mitochondria even when cytosolic NADH levels are low.

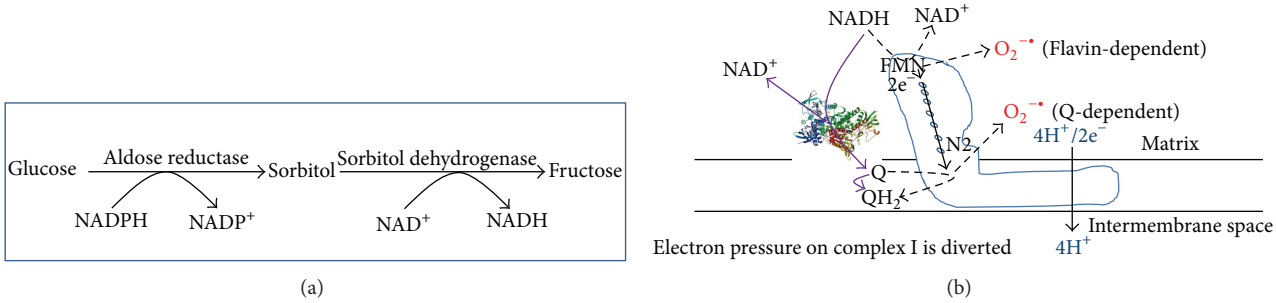

Figure 7: (a) The polyol pathway becomes a major pathway for NADH production under diabetic conditions. NADH is produced in the second reaction catalyzed by sorbitol dehydrogenase. (b) Proposed approach that may alleviate complex I NADH pressure and minimize superoxide production. This could be achieved by a molecule (a protein or a chemical) that can transport electrons from NADH directly to CoQ by bypassing complex I. As this bypass electron transport occurs without proton pumping, no superoxide should be produced, thereby facilitating NADH oxidation and minimizing superoxide production.

ROS production. Future studies should be directed toward exploring these strategies.

Finally, it should be pointed out that while ROS have been thought to be involved in impairment of β cell function and insulin secretion, no clear evidence that antioxidants lower blood glucose in clinical settings has been reported. Nonetheless, in animal models of diabetes induced by streptozotocin, many compounds, particularly those from plants and herbs, have been shown to be able to lower blood glucose by scavenging ROS and attenuating oxidative stress [109–118]. The hypoglycemic effects of these compounds in human diabetes, however, remain to be fully evaluated. Additionally,

it should also be pointed out that while both metformin and berberine have been shown to lower blood glucose levels by inhibiting complex I function [119–125], how they exert their actions on β cell complex I also remains to be investigated.

9. Summary

In this paper, we have summarized the glucose metabolic pathways and the roles of metabolic intermediates pyruvate and NADH in β cell function and insulin secretion. While the role of pyruvate recycling has been well established in β cell insulin secretion, the roles of NADH and complex I are yet to be fully elucidated. We thus focus our perspectives in this review on mitochondrial complex I that may contribute to redox balance under normal conditions and imbalance in diabetic conditions. We point out the fact that while complex I regulates NADH/NAD^+ recycling [126] and ROS production under physiological conditions [127], its role in diabetes whereby redox balance between NADH and NAD^+ is perturbed remains unexplored. We indicate that NADH overproduction due to chronic hyperglycemia would overload complex I, causing elevated levels of ROS production that has been previously postulated to contribute to the impairment of β cell function and insulin secretion [128–131]. Finally, we propose a hypothetic model of correcting this complex I-associated problem by alleviating complex I electron pressure that would also diminish complex I ROS production (Figure 7(b)). Future testing of this hypothesis may provide a potential therapeutic strategy for diabetes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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