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Insulin improves ß-cell function in glucose-intolerant rat models induced by feeding a high-fat diet

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ABSTRACT

Insulin therapy has been shown to contribute to extended glycemia remission in newly diagnosed patients with type 2 diabetes mellitus. This study investigated the effects of insulin treatment on pancreatic lipid content, and β-cell apoptosis and proliferation in glucose-intolerant rats to explore the protective role of insulin on β-cell function. A rat glucose-intolerant model was induced by streptozotocin and a high-fat diet. Plasma and pancreatic triglycerides, free fatty acids, and insulin were measured; and pancreatic β-cell cell apoptosis and proliferation were detected by a propidium iodide cell death assay and immunofluorescence for proliferating cell nuclear antigen. Relative β-cell area was determined by immunohistochemistry for insulin, whereas insulin production in pancreas was assessed by reverse transcriptase polymerase chain reaction. Islet β-cell secreting function was assessed by the index ΔI30/ΔG30. Glucose-intolerant rats had higher pancreatic lipid content, more islet β-cell apoptosis, lower β-cell proliferation, and reduced β-cell area in pancreas when compared with controls. Insulin therapy reduced blood glucose, inhibited pancreatic lipid accumulation and islet β-cell apoptosis, and increased β-cell proliferation and β-cell area in glucose-intolerant rats. Furthermore, impaired insulin secretion and insulin production in glucose-intolerant rats were improved by insulin therapy. Insulin can preserve β-cell function by protecting islets from glucotoxicity and lipotoxicity. It can also ameliorate β-cell area by enhancing β-cell proliferation and reducing β-cell apoptosis.

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1. Introduction

The natural history of type 2 diabetes mellitus is characterized by worsening hyperglycemia and progressive functional deterioration of the insulin-secreting pancreatic β-cells [1,2]. In the past decade, continued efforts have been made to target restoration or preservation of pancreatic β-cell function. Among all existing pharmacological interventions, short-term intensive insulin therapies, together with diet control, have been shown to contribute to extended glycemic remission without a need for any hypoglycemic agents in patients newly diagnosed with type 2 diabetes mellitus [3-8]. Weng et al [8] suggested that the restoration of first-phase insulin secretion is responsible for the improvement of β-cell function, which is represented by the homeostasis model assessment for β-cell function and acute insulin response after intensive insulin interventions. However, until now, the specific mechanism of restoration of β-cell function using insulin therapy remains unclear.

Along with insufficient pancreatic β-cell function, type 2 diabetes mellitus is often associated with central obesity and dyslipidemia [9,10], especially with the presence of circulating free fatty acids (FFAs) and triglycerides (TGs). Evidence emerging from the last few years indicates that FFAs and their derivatives not only act as metabolic substrates but play important roles in the pathogenesis of type 2 diabetes mellitus as well [11-13]. *β-Cell lipotoxicity* [14,15] refers to β-cell apoptosis resulting from chronic exposure to high FFA concentrations [16,17], which also contribute to decreased β-cell function [18]. Excessive lipid content in β-cells not only impairs glucose-stimulated insulin secretion [19] but also harms insulin production [20].

Glucotoxicity refers to the undermining of β-cell function by hyperglycemia [21,22]. Several studies have demonstrated β-cell apoptosis after long-term cell incubation with high glucose concentrations [23]. Apoptosis induction was also observed in human and *Psammomys obesus* pancreatic islets incubated with high concentrations of glucose [24-26]. In the pancreas, this glucotoxicity leads to progressive β-cell dysfunction [27], impaired insulin gene transcription [28], and a remarkable decrease in insulin secretion capacity [29,30].

Both glucotoxicity and lipotoxicity have been implicated in insulin resistance and β-cell failure in type 2 diabetes mellitus [31,32]. A deleterious interplay between glucotoxicity and lipotoxicity has been identified [32]. Whether the improvement of β-cell function by insulin therapy is due to the effects of ameliorating glucotoxicity and lipotoxicity remains uncertain at present. Combination of high-fat diet–fed and low-dose streptozotocin (STZ)–treated rats were used as the animal model for type 2 diabetes mellitus because this rat model simulates the natural history and metabolic characteristics of human type 2 diabetes mellitus and is also suitable for testing antidiabetic agents for the treatment of type 2 diabetes mellitus [33-36]. Therefore, the current study was designed to determine whether insulin treatment would eliminate glucotoxicity and lipotoxicity in pancreatic islets and improve β-cell function in glucose-intolerant animal models.

2. Materials and methods

2.1. Animal models

Male Wistar rats (180-200 g, 6-8 weeks old) were purchased from the experimental animal center of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (publication no. 85-23, revised 1996) and is approved by the Ethics Committee of Tongji Medical College. The animal model was prepared as before [34]. Briefly, rats were maintained at 22°C ± 2°C and subjected to a 12-hour/12-hour light/dark cycle. They were given free access to water and standard rodent feed diet (20% protein, 9% fat, and 53% carbohydrates; gross calorific value, 14 kJ/g) for 1 week. To induce diabetes mellitus, 25 mg/kg STZ (Sigma, St Louis, MO) in citrate buffer (pH 4.4) was administered to rats by tail vein injection according to a method by Reed et al [14]. The control group received only citrate buffer. Fourteen days after STZ treatment, rats were checked by oral glucose tolerance test (OGTT). Rats in the STZ-treated group whose area under the curve for glucose exceeded the mean + 2 SD of the control group were selected to continue with a high-fat diet. The high-fat diet consisted of 13% protein, 45% fat (mainly from the cooked lard), and 40% carbohydrates, with a gross calorific value of 20 kJ/g. After 2 months, development of diabetes was confirmed by measuring blood glucose. Rats with fasting glucose levels greater than 7.0 mmol/L or a 2-hour glucose level in OGTT greater than 11.1 mmol/L were considered to be glucose intolerant and used in the study.

2.2. Experimental design

Rats were divided into 4 groups: normal controls (NC, n = 10), glucose-intolerant rats fed with normal rodent feed (IN, n = 10), glucose-intolerant rats fed with high-fat feed (IH, n = 10), and glucose-intolerant rats fed with high-fat feed that also received insulin treatment (IHI, n = 10). For the IHI group, Humulin N (Lilly, Indianapolis, IN) was given by subcutaneous injection at a dose of 2 to 3 U/d between 5:00 and 6:00 PM. The Humulin N dose was adjusted every 3 days according to the glucose levels measured at 8:00 AM, so that the glucose levels of the IHI group were equivalent to those of the NC group. The other rats were injected with saline. After 10 weeks of treatment, rats were anesthetized with intraperitoneal injection of 80 mg/kg pentobarbital, blood samples were taken, and the pancreas were rapidly isolated. The tail of the pancreas was frozen in liquid nitrogen, whereas the remaining parts were fixed in 4% paraformaldehyde overnight and embedded in paraffin.

2.3. OGTT and insulin release test

One week before sacrificing the animals, rats were fasted overnight and given glucose solution (2 g/kg) by gavage. Blood samples were taken at 0, 60, 90, and 120 minutes after glucose administration from the tail vein. Samples for FFA analysis were placed in tubes containing 50 μL Paraoxon (diethyl-*p*-nitrophenyl-phosphate) (Sigma) diluted to 0.04% with

diethyl ether to prevent ex vivo lipolysis. Following immediate centrifugation at 4°C, plasma was separated and stored at –20°C until analysis. Incremental glucose (ΔG30) and insulin (ΔI30) responses were calculated as the difference between the values 30 minutes after and before glucose intake. The formula is as follows: $\Delta I30 / \Delta G30 = (I_{30} - I_0) / (G_{30} - G_0)$.

2.4. Plasma assays

Glucose was measured through the glucose oxidase method using glucose test kits (Beijing BHKT Clinical Reagent, Beijing, China). Insulin content was determined by radioimmunoassay using insulin radioimmunology kits (Tianjin Nine Tripods Medical and Bioengineering, Tianjin, China). Plasma TGs were measured with a GPO-Trinder reagent (Zhejiang Dongou Bioengineering, Zhejiang, China). Plasma FFA was determined by FFA kits (Nanjing Jiancheng Bioengineering, Nanjing, China).

2.5. Triglyceride and FFA measurements in pancreas

After weighing, frozen pancreas were homogenized in methanol/chloroform (1:1, vol/vol) with a tissue homogenizer. The extraction solution was transferred into a 1.5-mL Eppendorf tube and vibrated on the shaker at 4°C for 24 hours. The amounts of extracted TG and FFA were measured as described above and normalized to the weight of the pancreas.

2.6. Islet insulin content

The frozen pancreas were homogenized with a tissue homogenizer in 6-fold volume (6 mL of buffer solution per 1 g of tissue) of acidic ethanol buffer (1.5 mL 12 mol/L HCl in 100 mL 70% [vol/vol] ethanol) and incubated overnight at 4°C for further extraction. The next day, samples were centrifuged at 6000 rpm for 10 minutes at 4°C, and supernatants were collected and stored –20°C. The insulin level of supernatants was measured using insulin radioimmunology kits as described above and normalized to the weight of pancreas.

2.7. Reverse transcriptase polymerase chain reaction analysis

Total RNA was extracted from frozen pancreas using TRIzol solution (Promega, Madison, WI) according to the manufacturer's protocols. Complementary DNA was prepared from 3 μg total RNA by Moloney murine leukemia virus reverse transcriptase (Promega). The primers and polymerase chain reaction (PCR) conditions used for insulin (205 base pairs) were as follows: forward 5′-ACCTTTGTGGTCCTCACCTG-3′ and reverse 5′-GTGCAGCACTGATCCACAAT-3′; 1× (94 °C for 2 minutes); 32× (94°C for 1 minute, 60°C for 45 seconds, 72°C for 1 minute); and 1× (72°C for 10 minutes). The primers and PCR conditions used for actin (318 base pairs) were as follows: forward 5′-ACGAGGCCCAGAGCAAGA-3′ and reverse 5′-TTGGTTACAATGCCGTGTTCA-3′; 1× (94°C for 2 minutes); 30× (94°C for 40 seconds, 60°C for 1 minute, 72°C for 1 minute); and 1× (72°C for 10 minutes). The PCR products were separated on 1% to 2% agarose gels and visualized by ethidium bromide staining. The density of the bands photographed under UV illumination was analyzed by computer with Laboratory Work 4.0 (GSD8000 Protein scan systems, Gene, UVP, Cambridge, UK). The corrected relative value for the gene was expressed as a ratio of insulin to β-actin and then calculated as the mean ratio of insulin to β-actin.

2.8. Immunofluorescence

Five rats were used for immunofluorescence in each group, and 3 tissue sections for each rat were prepared as above. After washing with phosphate-buffered saline (PBS), sections were incubated with primary antibodies against proliferating cell nuclear antigen (PCNA, 1:50) and insulin (1:200) for 18 hours at 4°C. All antibodies were obtained from Santa Cruz Biotechnology, Paso Robles, CA. After three 5-minute washing steps with PBS, sections were incubated with Cy3-labeled goat anti-rat (Sigma) and fluorescein isothiocyanate–labeled (Boster, Wuhan, China) goat anti-mouse antibodies for 1 hour at room temperature. Fluorescence images were captured on a Nikon Digital Camera DXM1200 confocal microscope (Tokyo, Japan) at an optical depth of 1 μm.

2.9. Propidium iodide cell death assay

Tissue sections were prepared as above. Sections were incubated with 10 μg/μL RNase A (LSR-4875 Sigma) for 1.5 hours. After washing with PBS, sections were incubated with propidium iodide solution (Sigma) for 15 minutes. After washing, sections were stained with anti-insulin antibody (1:200, Santa Cruz Biotechnology) as previously described.

2.10. Assessment of apoptotic index and proliferation index

Five islets per tissue section were randomly chosen for assessment of apoptotic index (AI) and proliferation index (PI). The AI was calculated as the ratio of positive apoptotic β-cell number to the total cell number per islet, and the same counting method was used to determine the ratio of PCNA-positive cells per high-power field to calculate for the PI.

2.11. Immunohistochemistry

Three pancreatic sections were immunostained for insulin coupled with peroxidase detection (streptavidin-biotin complex [sABC] kits, Sigma). Briefly, tissue sections were deparaffinized with xylene, rehydrated with graded ethanol, and quenched in 0.3% H_2O_2/methanol solution for 10 minutes at room temperature to block endogenous peroxidase. After heating in a microwave with 0.01 mmol/L citric buffer (pH 6.0) for 20 minutes to recover the antigen, the sections were incubated for 5 minutes with 10% goat serum to block the background, followed by an overnight incubation with the primary antibody of rat insulin (1:100, Santa Cruz Biotechnology) at 4°C. Sections were labeled sequentially with biotinylated goat anti-mouse IgG for 1 hour at 37°C, followed by incubation with avidin-peroxidase complex. A color reaction was performed with 3,3′-diaminobenzidine. Finally, sections were counterstained in Mayer hematoxylin and mounted for light microscopic evaluation. Five islets were randomly chosen from each section under the microscope (magnification, ×400). The relative β-cell area was determined from the

ratio between areas occupied by insulin-positive cells and areas occupied by total pancreatic tissue. Densitometric analyses of the levels of insulin expression were performed using a quantitative image analysis system (MPIAS-1000, Wuhan Champion Image Technology, Wuhan, China).

2.12. Statistical analysis

All data were presented as mean ± SD. Comparisons were performed using 1-way analysis of variance followed by Student-Newman-Keuls multiple comparison test. Differences were considered statistically significant at $P < .05$. All data analyses were performed using the SPSS 10.0 package (SPSS, Chicago, IL).

3. Results

3.1. Plasma TG and FFA

Rats in the IN and IH groups showed higher plasma TG and FFA levels compared with the NC rats. However, plasma TG and FFA of the IH group increased significantly compared with those of the IN group ($P < .01$, Table 1). Insulin treatment did not show significant lowering effects on plasma FFA and TG ($P > .05$, Table 1).

3.2. Pancreatic TG and FFA content

A similar trend was observed for pancreatic TG and FFA as that in plasma. At the end of the study, both glucose-intolerant rats fed with normal and high-fat diet gained higher pancreatic TG and FFA content than controls ($P < .01$). In addition, the pancreatic TG and FFA levels of IH rats increased significantly compared with those of IN rats ($P < .01$). Interestingly, insulin treatment affected pancreatic TG and FFA differently than plasma. In insulin-treated glucose-intolerant rats, pancreatic TG and FFA remained close to the control levels despite a high dietary fat intake and decreased significantly compared with those of the IH and IN groups (Table 1).

Table 1 – Characteristics of plasma and islet lipids

Parameters	NC	IN	IH	IHI
Plasma TG (mmol/L)	0.35 ± 0.11	0.39 ± 0.13 *	1.02 ± 0.18 †,§	0.87 ± 0.14 †,§
Plasma FFA (mmol/L)	0.21 ± 0.07	0.31 ± 0.10 *	0.61 ± 0.12 †,§	0.49 ± 0.09 †,§
Islet TG (mmol/L)	9.96 ± 1.81	17.35 ± 4.75 †	24.58 ± 3.55 †,§	11.08 ± 2.22 ‡,¶
Islet FFA (mmol/L)	0.98 ± 0.11	1.81 ± 0.18 †	2.03 ± 0.16 †,‡	0.99 ± 0.15 §,¶

All values are mean ± SD (n = 10).
* $P < .05$. vs NC group.
† $P < .01$ vs NC group.
‡ $P < .05$ vs IN group.
§ $P < .01$ vs IN group.
¶ $P < .01$ vs IH group.

Table 2 – Plasma glucose concentrations (millimoles per liter) in OGTT

Group	$G_{0\ min}$	$G_{60\ min}$	$G_{90\ min}$	$G_{120\ min}$
NC	4.51 ± 0.79	8.85 ± 0.77	9.94 ± 0.44	5.81 ± 0.73
IN	7.74 ± 0.92 †	11.76 ± 1.14	12.71 ± 1.39	11.87 ± 1.49
IH	9.33 ± 0.52 †,§	13.39 ± 0.34	16.06 ± 0.52	14.59 ± 1.58
IHI	6.21 ± 1.05 †,§,¶	9.78 ± 0.69	10.96 ± 0.89	7.59 ± 0.66

All values are mean ± SD (n = 10).
† $P < .01$ vs NC group.
§ $P < .01$ vs IN group.
¶ $P < .01$ vs IH group.

3.3. OGTT results

Rats in the IN and IH groups showed higher plasma glucose levels compared with the NC rats ($P < .01$, Table 2). Insulin treatment dramatically reduced plasma glucose of rats in the IH group ($P < .01$).

3.4. Pancreatic ß-cell secreting function

β-Cell function was quantified as the ratio of the incremental insulin to glucose responses over the first 30 minutes of OGTT ($\Delta I30/\Delta G30$). Compared with that of the NC group, $\Delta I30/\Delta G30$ of the IN group decreased significantly ($P < .01$). Moreover, after the long-term lipid load, differences between the 2 glucose-intolerant groups were observed. The $\Delta I30/\Delta G30$ of the IH group was lower than that of the IN group ($P < .01$). However, after insulin treatment, $\Delta I30/\Delta G30$ improved dramatically, nearly reaching the level of the controls (vs IH group, $P < .01$; vs NC group, $P > .05$; Fig. 1).

3.5. Pancreatic insulin content and insulin gene expression

Parallel to the change in insulin expression of insulin detected by the sABC method, pancreatic insulin content

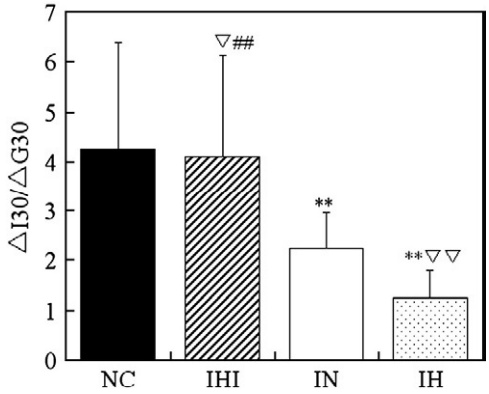

Fig. 1 – Insulin secretion evaluated by $\Delta I30/\Delta G30$. This test was done on both insulin-untreated and insulin-treated rats for 10 weeks. Incremental glucose ($\Delta G30$) and insulin ($\Delta I30$) responses were calculated as the difference between the values 30 minutes after and before glucose intake. The formula is as follows: $\Delta I30/\Delta G30 = (I_{30} - I_0)/(G_{30} - G_0)$. Data are presented as mean ± SD (n = 10).

and insulin messenger RNA (mRNA) levels in the IN and IH groups both notably decreased in comparison with those in the controls. A significant difference in pancreatic insulin content and insulin mRNA could also be seen between the IH and IN groups. Compared with the IH group, the pancreatic insulin content of the IN rats increased dramatically ($P < .01$). After insulin therapy, the pancreatic insulin content of the IHI rats reached levels similar to those found in normal rats ($P > .05$). In support of, insulin treatment resulted in a significant increase in insulin mRNA expression in the IH group compared with that in the IN group (without insulin treatment) ($P < .01$), although it was still at levels lower than those of the controls ($P < .01$) (Fig. 2).

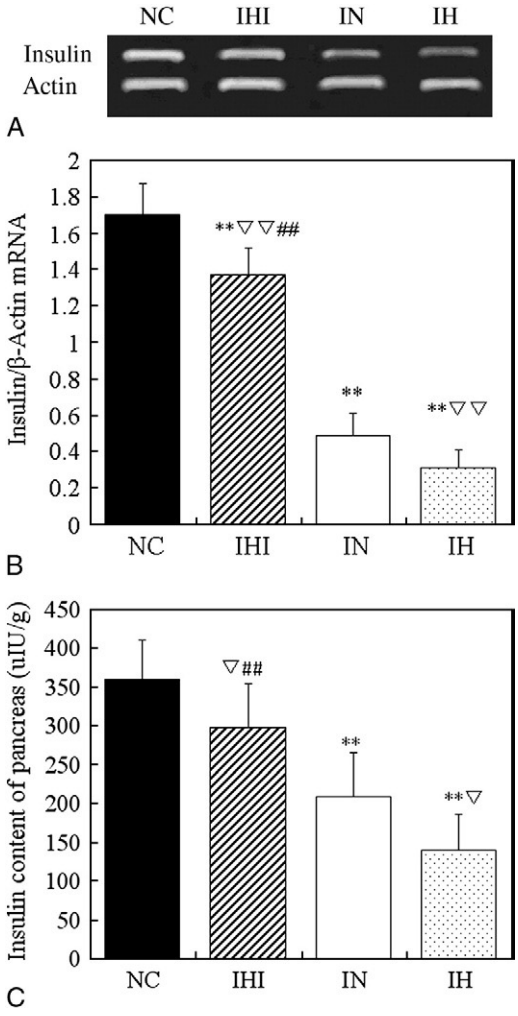

Fig. 2 – Islet insulin mRNA expression and insulin content in rats that were either nontreated or treated with insulin for 10 weeks. Five rats from each group were used for reverse transcriptase PCR and insulin content measurement. Reverse transcriptase PCR was performed to detect insulin and ß-actin expression (A). The corrected relative value for the gene was expressed as a ratio of insulin to ß-actin (n = 5) (B). The insulin level in the pancreas was measured using insulin radioimmunology kits and was normalized to the weight of pancreas (n = 5) (C).

3.6. *Islet ß-cell apoptosis and proliferation detection*

Pancreatic sections were double stained for insulin and propidium iodide. In glucose-intolerant rats fed with either a normal diet or a high-fat diet, many apoptotic islet β-cells were seen in each section, whereas only few apoptotic cells were seen in controls. Insulin treatment markedly inhibited islet β-cell apoptosis in glucose-intolerant rats fed with a high-fat diet. Proliferating cell nuclear antigen is a common proliferation indicator. Compared with that in controls, islet β-cell PCNA expression was significantly reduced in glucose-intolerant rats fed with a normal diet or a high-fat diet, and insulin significantly increased islet β-cell PCNA expression (Fig. 3D).

3.7. *Islet ß-cell area*

Densitometric analyses revealed that the islet β-cell area in the IH and IN groups were decreased by 21.1% and 38.8%, respectively, compared with the controls ($P < .01$). The IH group exhibited significantly lower insulin expression by islet cells compared with the IN group ($P < .01$). Insulin treatment markedly elevated the islet β-cell area of glucose-intolerant rats despite the high-fat diet, and islet insulin expression in the IHI group was 1.47-fold higher than that of the IH group ($P < .05$) and 1.18-fold higher than that of the IN group ($P < .01$). There was no significant difference in islet β-cell area between the IHI group and controls ($P > .05$) (Fig. 4).

4. Discussion

The present study demonstrated that insulin therapy at doses high enough to achieve reasonably good glycemic control markedly reduced islet fat content in glucose-intolerant rats and improved β-cell function. Pancreatic β-cell area in glucose-intolerant rats was ameliorated by insulin treatment through increased islet β-cell proliferation and diminished islet apoptosis. These indicate that insulin therapy contributes to the improvement of β-cell function and area by modulating glucose homeostasis and inhibiting the lipid accumulation on pancreatic islets.

Insulin therapy is usually instituted late in the course of type 2 diabetes mellitus, when glycemic control can no longer be maintained with oral antidiabetics. However, evidence from the past few years indicates that early implementation of a short course of intensive insulin therapy by continuous subcutaneous insulin infusion or multiple daily injections can induce sustained euglycemia in patients with type 2 diabetes mellitus [3-8]. Furthermore, Weng et al [8] reported that patients who maintained normoglycemia with intensive insulin therapy for 1 year showed greater recovery of β-cell function than patients administered with oral hypoglycemic agents. Thus, we infer that, in addition to the hypoglycemic effects of insulin, insulin itself is responsible for sustained normoglycemia.

High dietary fat intake is a strong risk factor for type 2 diabetes mellitus. It may lead to insulin resistance and β-cell dysfunction instituted by fatty acid species [37-40]. Generally, insulin inhibits lipolysis through activation of its downstream kinase PKB/AKT, resulting in inhibition of

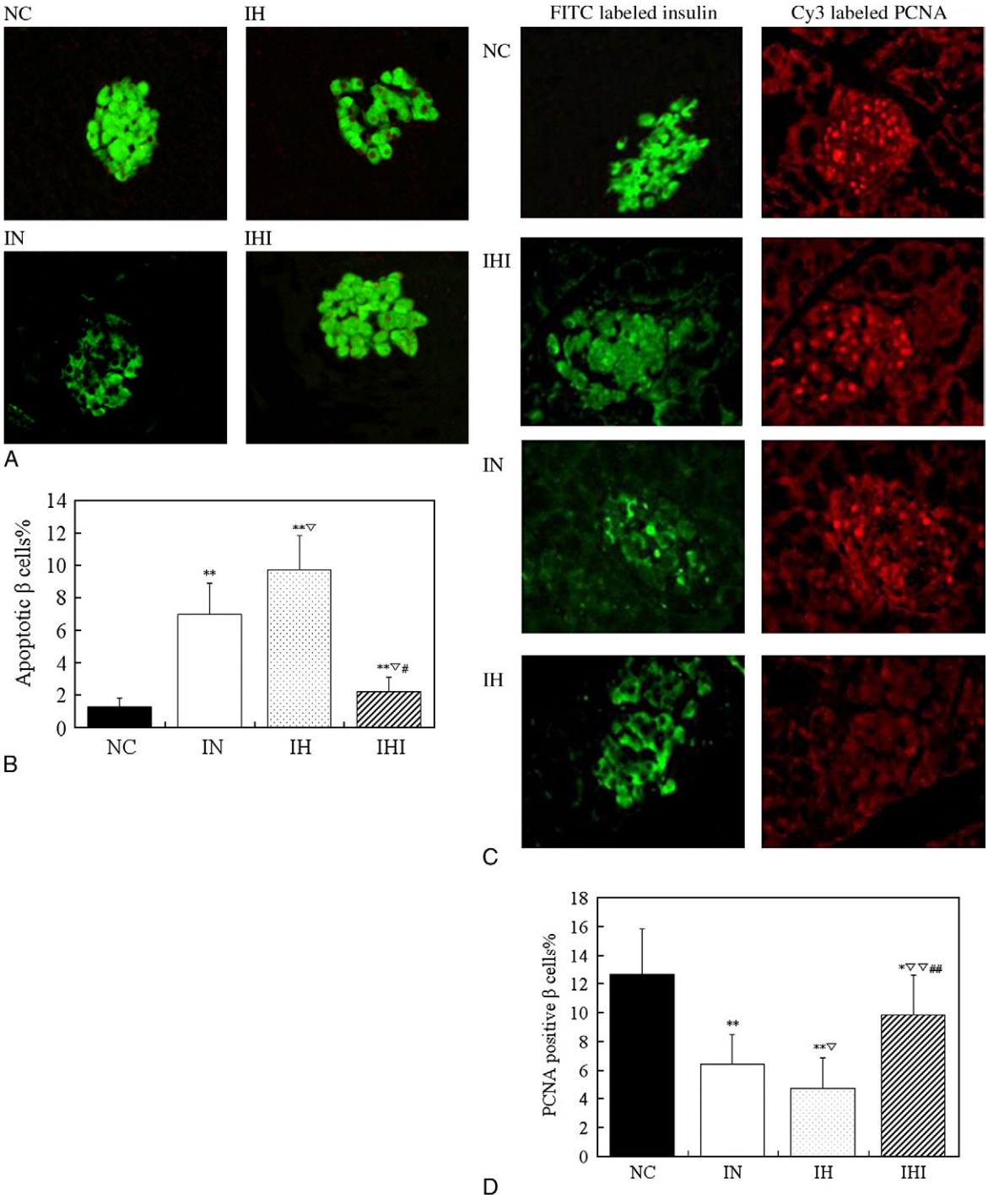

Fig. 3 – Islet ß-cell apoptosis and proliferation detection by propidium iodide cell death assay and immunofluorescence. Five rats from each group were used for islet ß-cell apoptosis and proliferation detection. For islet ß-cell apoptosis detection, pancreatic tissue sections were incubated with propidium iodide solution (red) and stained with anti-insulin antibody (green) (A). The AI was calculated as the percentage of positive apoptotic ß-cells per islet counted for each case (B). For islet ß-cell proliferation detection, Cy3-labeled PCNA (red) and fluorescein isothiocyanate–labeled insulin (green) images were captured on a confocal microscope (C). A PI was calculated for PCNA-positive cells (D).

protein kinase A and dephosphorylation of hormone-sensitive lipase. However, during insulin resistance, the lipolytic activity of insulin is reduced, which contributes to higher circulating FFA and ectopic storage of TG [41]. In our study, the increased islet TG and FFA levels in the IH group illustrate that the IH rats had more severe insulin resistance. This study demonstrated that insulin therapy can dramatically reduce islet FFA and TG content in glucose-intolerant rats exposed to high-fat feed, whereas it has only a mild effect on plasma FFA and TG levels. Free

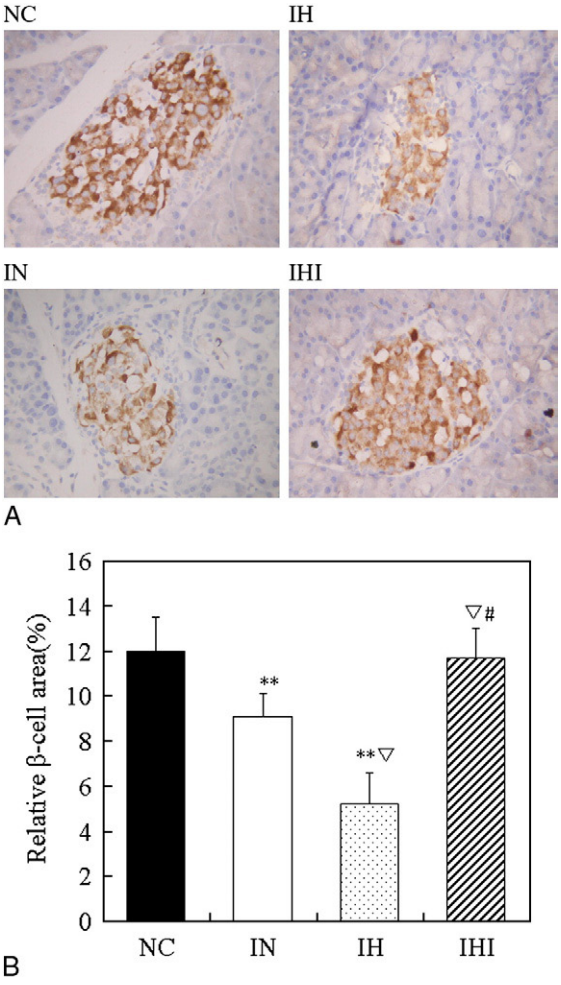

Fig. 4 – Islet insulin expression by sABC method and relative ß-cell area (percentage) in rats that were either nontreated or treated with insulin for 10 weeks. Five rats from each group were measured for insulin expression by sABC kits. Densitometric analyses of the levels of insulin expression were performed using a quantitative image analysis system (A). The relative ß-cell area was determined from the ratio between areas occupied by insulin-positive cells and areas occupied by total pancreatic tissue (B). *P < .05,P < .01 vs NC group; ▽P < .05, ▽▽P < .01 vs IN group; #P < .05, ##P < .01 vs IH group.**

fatty acids are essential β-cell fuel in the normal state but become toxic when chronically present in excessive levels. The results of this study showed that increases in islet TG and FFA contents in β-cells are associated with impairment of both insulin secretion and insulin protein production. This means that when the β-cell is challenged with glucose, insulin will not be secreted correspondingly. The potential mechanisms of insulin mRNA expression inhibition by islet FFA and TG include the negative regulation of pancreatic-duodenum homeobox-1 [42] and the activity of the insulin gene promoter [20]. Insulin therapy inhibited islet lipid accumulation, thus reversing the pancreatic β-cell dysfunction that resulted from lipotoxicity. However, whether the decrease in islet lipid levels is due to insulin itself or its lowering effects on glucose needs further research.

Glucose is an important physiological regulator of insulin secretion; and chronic hyperglycemia impairs glucose-induced insulin secretion and insulin gene expression [21], which is also defined as *glucotoxicity*. Although several strategies can be used to reduce hyperglycemia, insulin alone exhibits the strongest effect among them. This study demonstrated that insulin at doses high enough to achieve reasonably good glycemic control significantly increases insulin secretion as evaluated by ΔI30/ΔG30. Therefore, early intensive glycemic control plays a key role in the prevention of progressive β-cell dysfunction and worsening of diabetes, which has also been proven by several experimental and clinical studies [5,43-45].

Reduced β-cell area is a significant contributory factor in diminishing insulin secretion in type 2 diabetes mellitus. The balance of cell proliferation and death through necrosis or apoptosis determines net β-cell mass. Diminished proliferation, increased apoptosis, or both will result in a lower β-cell mass. This study showed that increased β-cell apoptosis and decreased β-cell proliferation in the glucose-intolerant rats on a normal or high-fat diet underlie the decreased β-cell mass in these animals. Insulin therapy partly reverses the above changes on β-cells and results in the recovery of β-cell area. In addition to inducing functional changes, insulin can also increase β-cell area by inducing proliferation and inhibiting cell apoptosis. Previous studies have reported that chronic hyperglycemia can induce β-cell apoptosis [25,26,46] and that increased FFA can be proapoptotic in β-cells [47]. This study found that insulin protected β-cells against these proapoptotic effects. Some molecules, including glucagon-like peptide–1 [48-50], epidermal growth factor, gastrin [51,52], and islet neogenesis-associated protein [44,53], have been shown to stimulate the proliferation of β-cells. Many of these factors have been shown to increase β-cell proliferation and expand β-cell mass. Nonetheless, these molecules including glucagon-like peptide–1 have not demonstrated strong hypoglycemic effects. The results of this study showed that insulin therapy enhances β-cell proliferation and reduces apoptosis. Thus, the possibility that insulin may contribute to regenerative actions through multiple pathways in human subjects with diabetes can provide new insights for regeneration therapy directed at pancreatic β-cells.

In summary, this study provides evidence that insulin treatment can protect pancreatic islets by regulating glucose and lipid metabolism, supporting the concept of interplay between FFAs and glucose metabolism. Furthermore, insulin may induce pancreatic β-cell proliferation and inhibit apoptosis, thereby increasing β-cell area and improving β-cell function. These findings, taken together with previous works, indicate that insulin management can preserve β-cell function and provide basis for early insulin treatment in diabetes. The mechanism of insulin preservation of pancreatic β-cells should be investigated in further studies.

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