Sitagliptin alleviated myocardial remodeling of the left ventricle and improved cardiac diastolic dysfunction in diabetic rats

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ABSTRACT

Objective: Sitagliptin, a dipeptidyl peptidase IV (DPP-IV) inhibitor, has a biological role in improving the serum levels of glucagon-like peptide 1 (GLP-1). Hence, we sought to determine the effect of sitagliptin on myocardial inflammation, collagen metabolism, lipid content and myocardial apoptosis in diabetic rats.

Materials and methods: The type 2 diabetic rat model was induced by low-dose streptozotocin and a high-fat diet. Characteristics of diabetic rats were evaluated by electrocardiography, echocardiography and blood analysis. Cardiac inflammation, fibrosis, cardiomyocyte density, lipid accumulation, and receptor-interacting protein kinase 3 (RIP3) level, related to apoptosis, were detected by histopathologic analysis, RT-PCR and western blot analysis to evaluate the effects of sitagliptin on myocardial remodeling of the left ventricle.

Results: Diabetic rats showed myocardial hypertrophy or apoptosis, inflammation, lipid accumulation, myocardial fibrosis, elevated collagen content, RIP3 overexpression, and left-ventricular dysfunction.

Sitagliptin could reverse the overexpression of RIP3 and alleviate cellular apoptosis in myocardial tissues. It could significantly improve left-ventricular systolic pressure and $+\text{dp/dt max}$, reduce the $E/E_0$ ratio, left ventricular end diastolic pressure, $-\text{dp/dt max}$ and Tau in diabetic rats.

Conclusions: Sitagliptin might have a myocardial protective effect by inhibiting apoptosis, inflammation, lipid accumulation and myocardial fibrosis in diabetic rats, for a potential role in improving left-ventricular function in diabetes.

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1. Introduction

Accumulating evidence indicates that diabetic cardiomyopathy (1,2) is a disorder of myocardial lesions induced by high glucose. It features myocardial hypertrophy or apoptosis (3), inflammation, lipid accumulation, and myocardial fibrosis (4). As well, it might feature systolic and/or diastolic dysfunction of the heart and increased mortality (5). Sari et al. (6) declared that cardiac endoplasmic reticulum stress (ERS) and ERS-initiated apoptosis were involved in diabetes. Receptor-interacting protein 3 (RIP3), an important signal molecule, is involved in tumor necrosis factor $\alpha$ (TNF-$\alpha$)-mediated apoptosis and related to mitochondrial energy metabolism (7,8), but its expression in the diabetic heart is not known.

Sitagliptin is a dipeptidyl peptidase IV (DPP-IV) inhibitor and has a role in improving the activity of glucagon-like peptide 1 (GLP-1) (9), an incretin hormone secreted from the distal intestine L cells with glucose-dependent release that promotes the secretion of postprandial insulin (10). GLP-1 receptors (GLP-1Rs) exist widely in islets, myocardium and brain tissue. Relatively low GLP-1 concentrations as compared with $\text{in vivo}$ blood concentrations promoted insulin secretion independent of the cAMP-protein kinase A pathway (11). With decreased activity of
GLP-1 in diabetic patients, endogenous GLP-1 could be dissolved rapidly by DPP-IV (12).

Sitagliptin could prevent GLP-1 degradation in the short term, elevate serum levels of GLP-1 and enhance or delay the release of insulin, to lower blood glucose (13,14). Furthermore, recent reports demonstrated that GLP-1 has a key role in the inhibiting apoptosis during ischemia-reperfusion (I/R) injury and improved cardiac function (15).

Hence, we evaluated the effect of sitagliptin on inflammation, collagen metabolism, lipid content, myocardial apoptosis (RIP3 expression) and cardiac function in diabetic rats to detect additional benefits for sitagliptin beyond its blood glucose-lowering effect.

2. Materials and methods

2.1. Animals and supplementation

We purchased 70 male, 8-week-old Wistar rats (120–140 g) from the experimental animal center of Shandong University (Jinan, China). All experimental procedures were performed in accordance with animal protocols approved by the Shandong University Animal Care Committee. Rats were housed at 22 °C with 12-h light/dark cycles. All mice were fed a high-fat diet (34.5% fat, 17.5% protein, 48% carbohydrate; Beijing HFK Bio-Technology). Four weeks later, the intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPTT) were performed and blood was sampled by the jugular vein. Fasting insulin and fasting blood glucose (FBG) were measured, and the insulin sensitivity index [ISI = ln (FBG × fasting insulin)]−1 was calculated. Control rats received citrate buffer (intraperitoneally) alone and rats with insulin resistance received a single intraperitoneal injection of STZ (Sigma, St. Louis, MO; 30 mg/kg intraperitoneally in 0.1 mol/L citrate buffer, pH 4.5) as described (16). At one week after STZ administration, rats with FBG > 11.1 mmol/L in 2 consecutive analyses were considered the diabetic model. Animals were then randomized to one of 2 groups for treatment: control rats (n = 10), normal chow; or diabetes rats, induced with DPP-IV inhibitor (low-dosage sitagliptin, 30 mg/kg/d) (n = 20) [DPP-IV (Low)] and (high-dosage sitagliptin, 50 mg/kg/d) (n = 20) [DPP-IV (High)]. Treatment with sitagliptin started 4 weeks after STZ injection. The rats were killed after 17 weeks of diabetes.

2.2. Blood analyses

Blood was collected from the jugular vein after rats fasted for 8 h. FBG and circulating levels of cholesterol, triglycerides, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were analyzed with use of the Bayer blood chemistry analyzer (Bayer, Tarrytown, NY). Free fatty acid (FFA) concentrations were measured by use of an enzymatic test kit (CSB-E08770r; HuaMei Bio-TECH, Wuhan, China). ISI was calculated. Plasma insulin levels were measured by use of the rat insulin ELISA kit based on the direct Sandwich ELISA technique (Mercodia AB, Sweden). Hemo-globin A1C (HbA1c) level was measured by the NycoCard Reader (Ranbaxy, India).

2.3. Electrocardiography and echocardiography

ECG was recorded with the limb leads (I, II, III, aVR, aVL, aVF) to evaluate cardiac electrical activity. Echocardiography involved use of the Philips IE33 imaging system (S12–4). Images were obtained from 2D, M-mode, pulsed-wave Doppler and tissue Doppler imaging (TDI). All measurements were performed by the same researcher and were averaged from 6 consecutive cardiac cycles. Wall thickness and LV dimensions, including LV end systolic diameter (LVEDs) and LV end diastolic diameter (LVEDd), were obtained from the long-axis view. LV ejection fraction (LVEF) and fractional shortening (FS) were measured according to American Society of Echocardiography guidelines (16,17). The mitral-valve pulsed Doppler recordings were obtained from the apical four-chamber view. After pulsed Doppler, transmitral flow velocity variables, including peak E, peak A and E/A ratio were evaluated (the early (E') and late (A') diastolic velocity were analyzed and E'/ A' and E/E' values were calculated) and tissue Doppler imaging of the mitral annulus was obtained from the apical four-chamber view.

2.4. Hemodynamics by Millar catheter

Rats were anesthetized with urethane (1 g/kg), then the right carotid artery was cannulated and a transducer was advanced into the left ventricle to measure the rate of pressure changes (±dp/dt max), blood pressure, and heart rate with a micropipet transducer (Millar Instruments) and LVED pressure (LVEDP) was measured. Tau was calculated with the LV time constant Tau = P/(-dp/dt).

2.5. Morphometric analysis

Heart tissues were fixed in 10% formalin, embedded in paraffin, and sectioned at 5-mm thick. A single myocyte was measured with images captured from H&E-stained sections. The myocyte cross-sectional area was assessed by ×400 magnification within the LV, and a mean was obtained by quantitative morphometry with automated image analysis (Image-Pro Plus 5.0; Media Cybernatics, Houston, TX).

Dark green-stained collagen fibers were quantified to measure fibrosis in Masson trichrome-stained sections. The collagen volume fraction (CVF) and perivascular collagen area to luminal area (PVCA/LV) were analyzed by quantitative morphometry with automated image analysis (Image-Pro Plus 5.0). CVF was calculated as reported previously (18). Perivascular collagen was excluded from the CVF measurement. To normalize the PVCA around vessels with different sizes, perivascular collagen content was represented as the PVCA/LV ratio. Interstitial and perivascular fibrosis was evaluated by Picrosirius red staining. Sections were stained with 0.5% Sirius red (Sigma) in saturated picric acid for 25 min. Collagen was stained red. Myocardial frozen sections (5 mm) were stained with Oil-red O (Sigma) for 10 min, washed, then counterstained with hematoxylin for 30 s. A Nikon microscope (Nikon, Melville, NY) was used to capture images.

An in situ cell death detection kit (Roche GmBH, Germany) was used for TUNEL assay. Briefly, slides were deparaffinized, rehydrated with xylene; underwent a graded ethanol series; and were permeabalized with hot 0.1 M citrate buffer, pH 6.0; incubated with reaction mixture containing TdT; and labeled with dUTP for 1 h at 37 °C. Images were captured by confocal laser scanning microscopy (Zeiss LSM510). For a negative control, TdT was omitted from the reaction mixture.

2.6. Immunohistochemical staining

Paraffin sections underwent immunohistochemistry by a microwave-based antigen retrieval method. Sections were incubated with primary antibodies for rabbit polyclonal collagen I and III, tumor necrosis factor TNF-α, and interleukin 6 (IL-6) (Abcam, Cambridge, MA) overnight, then with biotinylated secondary antibody for 30 min at 37 °C. Negative controls were omission of the primary antibody. Stained sections were developed with diaminobenzidine and counterstained with hematoxylin. Sections
A

B
were viewed under a confocal FV 1000 SPD laser scanning microscope (Olympus, Japan).

2.7. RT-PCR

Total RNA was prepared with the TRIzol reagent (Gibco/Invitrogen, Carlsbad, CA). RT-PCR involved the primer sequences for RIP3, forward 5'-ACC ACTGAGCGAGCATCCTTCC-3', and reverse 5'-CCCGGAACACGGCTCCGAAC-3'; and β-actin, forward 5'-AGA CCT TCA ACA CCC CAG-3' and reverse 5'-CAC GAT TTC CCT CTC AGC-3'. Reactions were carried out on a real-time PCR thermocycler (IQ5 Real-Time PCR cycler; Bio-Rad), with SYBR green as fluorescence dye. Relative expression analysis involved the $2^{-\Delta\Delta CT}$ method.

**Fig. 1.** (continued).

![Fig. 1](image_url)

**Fig. 1.** Blood analysis demonstrating metabolic disturbance. **A.** FBG: fasting blood glucose; BG: at 10:00 am; HbA1c: Glycated hemoglobin; FINS: Fasting insulin; ISI: insulin sensitivity index. **B.** TC: Total cholesterol levels; TG: triglyceride levels; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; FFAs: free fatty acids. **C.** Fasting serum levels of GLP-1 and serum levels of GLP-1 at 30 min after oral glucose testing. **D and E.** Intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) findings. (Data are mean ± SEM; n = 7–10 per group. Compared with the control group, *P < 0.05, **P < 0.01; Compared with DPP-IV (High), #P < 0.05, ##P < 0.01; Compared with DPP-IV (Low), xP < 0.05, xxP < 0.01.)
2.8. Western blot analysis

Western blot analysis was performed as described previously (19) with antibodies for RIP3 (Calbiochem, La Jolla, CA), collagen I and III, TNF-α and IL-6 (Abcam), followed by anti-IgG horseradish peroxidase–conjugated secondary antibody. Protein levels were normalized to that of β-actin as an internal control and phospho-specific proteins to that of total protein.

2.9. Statistical analysis

Data are presented as mean ± SD for continuous variables or proportions. After testing for normal distribution of variables, data were analyzed by Student’s two-tail t test and one-way ANOVA followed by post-hoc least significant difference test as appropriate. Correlation was assessed by Pearson or Spearman rank order correlation test, as appropriate. Statistical analyses involved use of SPSS 16.0 (SPSS, Chicago, IL, USA). A two-tailed P < 0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics of diabetic rats

Diabetic rats showed polydipsia, polyphagia, and polyuria as compared with other rats (P < 0.01). As compared with the control
group, diabetic rats showed decreased body weight ($P < 0.01–0.05$) and heart rate and increased blood pressure ($P < 0.01–0.05$); however, with sitagliptin treatment, body weight increased and heart rate and blood pressure decreased ($P < 0.01–0.05$).

3.2. Sitagliptin improved blood glucose and lipid metabolism

Insulin resistance appeared after 4 weeks with a high-fat diet. One week after STZ injection, FBG increased markedly in diabetic rats. Diabetic rats showed insulin resistance, moderate hyperglycemia, hyperlipidemia, and decreased ISI ($P < 0.05$) (Fig. 1A). Serum levels of total cholesterol, total triglycerides, LDL-C, and FFA were higher for diabetic rats than controls ($P < 0.05$) and decreased with sitagliptin treatment ($P < 0.01–0.05$) (Fig. 1B). Plasma GLP-1 decreased under both fasting and 30 min after oral glucose ($P < 0.01–0.05$) and increased with sitagliptin treatment ($P < 0.01–0.05$) in diabetic rats (Fig. 1C). Sitagliptin had no effect on IPGTT and IPITT results (Fig. 1D and E).
3.3. Sitagliptin improved heart features

Control rats showed P waves, T waves and amplitude consistency of QRS waves (Fig. 2.1). For diabetic rats, 66.7% showed abnormal ECG results, and QRS "electric alternans" phenomena were slightly more common than in controls rats, with supraventricular tachycardia, ventricular tachycardia, slow heart rate and other complex arrhythmia in diabetic rats. However, with sitagliptin treatment, for diabetic rats, 42.4% showed abnormal ECG results, with no complex arrhythmia.

3.4. Sitagliptin improved LV structure and function

At the beginning, the size of LV chambers, LVIDs and LVIDd did not differ in control and diabetic rats ($P > 0.05$); however, at the end, compared with controls, diabetic rats showed increased LVIDs and LVIDd ($P < 0.05$) (Fig. 2.2). Sitagliptin treatment decreased LVIDs and LVIDd ($P < 0.05$).

At the beginning, control and diabetic rats showed no diastolic or systolic dysfunction ($P > 0.05$). However, at the end, compared with controls, diabetic rats showed decreased LVEF and FS ($P < 0.01$–0.05), which was increased with sitagliptin treatment ($P < 0.01$–0.05) (Fig. 2.2). Compared with controls, from 13 to 21 weeks, E/A, $E'/A'$ decreased ($P < 0.01$–0.05) and $E/E'$ increased ($P < 0.01$–0.05) in diabetic rats, which was reversed with sitagliptin treatment (Fig. 2.2).

Compared with controls, diabetic rats showed decreased LVSP ($P < 0.01$) and $+dp/dt\ max$ ($P < 0.01$–0.05) and increased LVEDP ($P < 0.01$); however, sitagliptin reversed these findings (Fig. 2.3).
Fig. 4. Expression of glucagon-like peptide 1 (GLP-1) and receptors in the heart. Expression of A: GLP-1 and B: GLP-1 receptor. (Data are mean ± SEM; Compared with control, *P < 0.05, **P < 0.01; Compared with DPP-IV (High), #P < 0.05, ##P < 0.01; Compared with DPP-IV (Low), xP < 0.05, xxP < 0.01.).

Fig. 5. DPP-IV inhibitor sitagliptin alleviated cardiac fibrosis in diabetic rats. A1: Masson trichrome staining (collagen is blue and myocardium red) and Picrosirius red staining—collagen fibers stained bright red; bright-field (A2), dark-field (A3) show myocardial interstitial fibrosis. A4–A5: Picrosirius red staining—bright-field (A4), dark-field (A5) show perivascular fibrosis. Quantitative analysis of B: CVF, C: collagen content by hydroxyproline assay, and D: perivascular collagen area to luminal area (PVCA/LA). (Data are mean ± SEM; Compared with control, *P < 0.05, **P < 0.01; Compared with DPP-IV (High), #P < 0.05, ##P < 0.01; Compared with DPP-IV (Low), xP < 0.05, xxP < 0.01.).
3.5. Sitagliptin reversed the size of both the heart and cardiomyocytes

Compared to controls, diabetic rats showed larger heart sizes, higher ratio of heart weight to body weight (HW/BW) \( (P < 0.05) \) (Fig. 3A), and increased myocardial hypertrophy and cellular cross-sectional area \( (498.37 \pm 14.31 \text{ vs } 347.84 \pm 12.27 \, \mu\text{m}^2, \, P < 0.01) \) (Fig. 3A–C). Diabetic rats also showed myocardial cell hypertrophy, muscle fiber derangement or fracture, irregular shape, and uneven size of nuclei. Sitagliptin treatment reversed these findings.

3.6. Sitagliptin increased GLP-1 content and downregulated the density of GLP-1 receptors in diabetic hearts

As compared with controls, diabetic myocardial tissue showed decreased GLP-1 content \( (P < 0.01) \) (Fig. 4A) and expression of GLP-1 receptor (GLP-1R) \( (P < 0.01) \) (Fig. 4B), which were reversed with sitagliptin treatment \( (P < 0.01) \).

3.7. Sitagliptin alleviated cardiac fibrosis in diabetic rats

The diabetic heart showed cardiac fibrosis, with a diffuse, small, patchy, and nonuniform pattern, as well as destroyed and disorganized collagen network structure in the interstitial areas (Fig. 5A1–A3) and perivascular areas (Fig. 5A4–A5). CVF \( (4.8 \pm 0.49 \text{ vs } 0.47 \pm 0.06, \, P < 0.01–0.05) \) and collagen content \( (15.8 \pm 0.43 \text{ vs } 7.06 \pm 0.31 \, \mu\text{g/mg}, \, P < 0.01) \) were higher in diabetic rats than controls (Fig. 5B and C), and perivascular collagen area/lumen area ratio (PVCA/LA) was increased \( (2.74 \pm 0.37 \text{ vs } 0.5 \pm 0.02, \, P < 0.01–0.05) \) (Fig. 5D). Compared with controls, diabetic rats showed increased expression of collagen I and III (Fig. 6.1). These findings were reversed with sitagliptin treatment.
3.8. Sitagliptin downregulated the expression of MMP-1 and 9, TNF-α and IL-6 in diabetic hearts

Compared with controls, diabetic rats showed increased expression of MMP-1 and -9 and TNF-α and IL-6 (Fig. 6.2 and 6.3), which was decreased with sitagliptin treatment.

3.9. Sitagliptin reduced lipid accumulation in myocardial and epicardial tissue in diabetic hearts

The accumulation of triglycerides caused myocardial fatty degeneration in diabetic rats, with increased staining in myocardial tissue (7.03 ± 0.31 vs 0.32 ± 0.01, P < 0.01) (Fig. 6.4) and epicardial adipose tissue (12.76 ± 0.48 vs 0.32 ± 0.01, P < 0.01), which was reduced with sitagliptin treatment (P < 0.01–0.05).

3.10. Sitagliptin downregulated the expression of RIP3 in diabetic hearts

Compared with controls, diabetic myocardial tissues showed increased number of RIP3-positive particles distributed around the nucleus, which were decreased in number with sitagliptin treatment (Fig. 7.1). The mRNA and protein levels of RIP3 were increased in diabetic myocardial tissues (P < 0.01–0.05) and were decreased with sitagliptin treatment (P < 0.01–0.05).

3.11. Sitagliptin inhibits myocardial apoptosis

Diabetic rats showed irregular myocardial nuclei, nuclear pyknosis, chromatin aggregation, early presentation of apoptotic cells, with clear myocardial cellular nucleolus, smooth and complete nuclear membrane in controls and no obvious aggregation of...
chromatin, no nuclear pyknosis and nuclear membrane still intact; sitagliptin treatment smoothed the nuclear membrane in diabetic rats (Fig. 7.2). Compared with controls, diabetic rats showed increased proportion of apoptotic cells ($P < 0.01$), which was decreased with sitagliptin treatment ($P < 0.01$). With the correlation between the RIP3 mRNA and cellular apoptotic rate in diabetic myocardial tissues (Pearson $r = 0.795$, $P < 0.001$), the positive percentage of apoptosis was related to RIP3 mRNA in diabetic myocardial tissues.

4. Discussion

Sitagliptin, a DPP-IV inhibitor, has a biological role in improving the serum levels of GLP-1. GLP-1 reduces hyperglycemia but may also trigger direct effects on the heart (20). We sought to determine the effect of sitagliptin on myocardial inflammation, collagen metabolism, lipid content and myocardial apoptosis in diabetic rats. Sitagliptin improved metabolic disturbance, reversed myocardial remodeling and lipid accumulation, inhibited cardiac inflammation, reduced collagen synthesis, and minimized apoptotic cells in STZ-induced diabetic rat hearts. RIP3 was overexpressed and positively correlated with number of apoptotic cells in diabetic myocardial tissues; sitagliptin downregulated the expression and minimized the number of apoptotic cells. Sitagliptin could improve the systolic and diastolic functions of the left ventricle in diabetic rats by reducing the $E/E'_{0}$ ratio, promoting LVSP and decreasing LVEDP, $±dp/dt$ max and Tau. It reversed the altered blood glucose, HbA1 and lipid levels, and plasma GLP-1 and insulin levels, and reduced fat deposition in diabetic myocardial and epicardial tissues, so sitagliptin may play an essential role in improving diabetic cardiac dysfunction and cardiomyopathy.
Inflammation is a key pathophysiologic factor in diabetic cardiomyopathy (1,2,16,21), and is closely related to fat deposition. Ti et al. (16) reported that TNF-α and IL-6 were overexpressed in diabetic rats. Biomarkers of inflammation including TNF-α and IL-6 levels are associated with risk of developing type 2 diabetes. Consistent with these reports, our results showed an increase in TNF-α and IL-6 levels in diabetic myocardial tissues as well as fat deposition in diabetic myocardial and epicardial tissues. However, sitagliptin could reduce the expression of TNF-α and IL-6 and fat deposition in the diabetic heart, so sitagliptin might lower the morbidity and mortality of diabetic complications associated with inflammation (22,23).

Previous studies (1,2,16) have demonstrated that myocardial fibrosis is a typical characteristic of diabetic cardiomyopathy. Both the deposition and disordered arrangement of type I and/or III collagen and abnormal expression of MMPs/TIMPs, and cytokines TGF-β1 or insulin-like growth factor 1 contribute to the development of myocardial fibrosis and cardiac diastolic dysfunction. However, some reports (24,25) demonstrated that a GLP-1R agonist can inhibit the expression of TGF-β1. However, this study found that sitagliptin reduced aberrant interstitial and perivascular collagen accumulation, as well as total collagen content. Echocardiography and catheterization results revealed that the LV diastolic dysfunction improved, which was attributed...
to decreased cardiac fibrosis, reduced collagen I and III content and MMP-1 and -9 expression. Thus, sitagliptin improved LV diastolic dysfunction in diabetic rats by attenuating myocardial fibrosis.

In addition to the above changes, diabetic myocardial tissues show apoptosis and necrosis of myocardiocytes. Recent reports (26–29) demonstrated that GLP-1 could play a key role in the progress of inhibiting apoptosis during I/R injury. In contrast, glibenclamide exacerbates I/R injury and deteriorates cardiac function (30,31). A GLP-1R agonist enhanced β-cell proliferation and neogenesis in STZ-treated rats and reduced β-cell apoptosis in part via endoplasmic reticulum stress (32,33). DeNicola et al. (34) found that GLP-1R served as a novel approach to eliciting cardioprotection and mitigating oxidative stress-induced injury. Consistent with these reports, our results show that sitagliptin minimized the number of apoptotic cells and RIP3 was overexpressed and correlated positively with the number of apoptotic cells in the diabetic rat heart; furthermore, sitagliptin downregulated the expression of RIP3 and minimized the number of apoptotic cells. Attenuating cardiac apoptosis might be due to downregulated RIP3 after administration of sitagliptin. Thereafter, sitagliptin improved LV systolic dysfunction. An GLP-1 analog protected cardiomyocytes against high glucose-induced apoptosis by activating the Epac-1/Akt pathway (35). However, the exact mechanism of sitagliptin reversing these changes and the signal pathway remains unknown.

In summary, our study is a first step in unifying many factors in the diabetogenic processes in a relevant in vivo system and investigates sitagliptin as a DPP-IV inhibitor for the diabetic heart to help move us closer to finding effective treatment or prevention strategies. Future study could investigate difference doses of sitagliptin.

Fig. 7. Sitagliptin reduced the expression of RIP3 and inhibited myocardial apoptosis. Sitagliptin reduced the expression of 7.1(A–C): RIP3; 7.2(A–B): inhibited myocardial apoptosis; and 7.2(C): proportion of myocardial apoptotic cells positively correlated with relative levels of RIP3 mRNA (Data are mean ± SEM; Compared with control, *P < 0.05, **P < 0.01; Compared with DPP-IV (High), #P < 0.05, ##P < 0.01; Compared with DPP-IV (Low), ¥P < 0.05, ¥¥P < 0.01).
Conflict of interest

The authors declare that they have no competing interests.

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Yu-Sheng Liu, wrote the manuscript and researched data. Zhi-Wei Huang, is studying for her doctorate (her tuter Prof. Xiu-Li Ju) in the Shandong University and also is working in the Department of Hematology, The Qilu Children’s Hospital of Shandong University, performed the animal model and molecular analysis. Lin Wang and Xin-Xin Liu reviewed/edit the manuscript, and Yong-Mei Wang performed echocardiology. Yun Zhang and Mei Zhang, researched data and reviewed/edit the manuscript. The study was supported by the National Basic Research Program of China (973 Program, 2011CB503906) and the National Science Foundation of China (No. 81270404,81470559) and by the Seed Fund (grant S2013).

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