Induction of Caveolin-3/RyR2 By Liraglutide Ameliorates Diabetic Cardiomyopathy

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Original investigation

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Induction of caveolin-3/RyR2 by liraglutide ameliorates diabetic cardiomyopathy

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Abstract

Background

Liraglutide (LIRA), a Glucagon-like peptide-1 receptor agonist (GLP-1RA), showed potent cardioprotective effects with the mechanism remained incompletely understood. Caveolin-3 (Cav3) is the cardiomyocytes specific caveolae structural protein, decreased in the diabetic heart. Therefore, this study aimed to investigate whether LIRA exerts its effect on cardiac function in rats with type 2 diabetes mellitus (T2DM) via enhance Cav3 expression.

Methods

T2DM rats were used as study subjects and randomly divided into four groups: 1) CON group, 2) CON+L group, 3) DM group and 4) DM+L group. All rats received either saline or LIRA 0.2 mg/kg (by i.p injection) per day for 4 weeks. After the model was successfully established, cardiac function was determined by invasive hemodynamic evaluation methods. Immunohistochemistry and western blot were performed to understand the molecular mechanism between cardiac function and LIRA.

Results

Based on our results, DM group displayed higher blood glucose than Con group (20.57±2.75 mol/L vs. 4.34±0.21 mol/L), while blood glucose level in DM+L group was lower than DM group after received LIRA (10.36±1.84 mol/L). LVSP (91.39±4.98 mmHg), LV +dp/dtmax (4040.74±197.72 mmHg/s) were significantly reduced in DM group, and diabetic rats also exhibited reduced -dp/dtmax (2926.5±142.3 mmHg/s) and elevated LVEDP (10.87±0.83 mmHg). LIRA treatment showed a trend to enhance LVSP (110.76±5.61 mmHg) and ± dp/dtmax (5860.41±200.32 mmHg and 3996.8±179.3 mmHg), decreased LVEDP (7.23±0.58 mmHg). The expression of Cav3, eNOS and RyR2 was significantly decreased in the myocardium in DM group, which increased in DM+L group after LIRA administrated. Hemodynamic data showed DM rats exhibited impairment of myocardial function, while LIRA improved cardiac systolic and diastolic function, attenuate diabetic cardiomyopathy injury by improving Cav3/eNOS/NO signaling, reducing ROS level in cardiac tissues, and increasing interaction of Cav3 and ryanodine receptor 2 (RyR2).

Conclusions

Liraglutide ameliorates cardiac dysfunction in rats with type 2 diabetes mellitus via reducing ROS level in cardiac tissues, improving Cav3/eNOS/NO signaling and increasing interaction of Cav3 and RyR2.

Keywords Type-2 diabetes Mellitus, liraglutide, caveolin-3, ryanodine receptor2, myocardial dysfunction

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Background

Patients with diabetes mellitus have >2× the risk for developing heart failure[1]. T2DM carries risks of both cardiovascular (CV) and microvascular complications. Cardiomyopathy is manifested by various structural and functional anomalies in the myocardium, such as cardiomyocyte hypertrophy, myocardial fibrosis, cardiac autonomic neuropathy, and apoptosis, as well as left ventricular diastolic and systolic dysfunction[2]. Given the prevalence of cardiovascular disease in this population, a complete understanding of the cardiovascular safety and efficacy of glucose-lowering drugs is needed[3]. Hence, the discovery of new pharmacological targets or the development of novel therapeutic strategies to treat cardiomyopathy of diabetes is paramount.

Caveolae are a specialized type of lipid raft that is stabilized by oligomers of the caveolin protein. Within these microdomains, caveolins interact with numerous signaling molecules such as endothelial nitric oxide synthase（eNOS）, PI3K, and MER/MRK etc. That are required in cardiac protection initiated by a variety of cardioprotective interventions[4, 5]. Cav3, the dominant isoform of cardiomyocyte caveolae, is reduced in diabetic hearts in which oxidative stress is increased[6].

Accumulating evidence has revealed that excessive reactive oxygen species (ROS) generation, impaired eNOS/NO, and cell apoptosis contribute to diabetes-induced myocardial injury [7, 8]. Dysfunctional calcium release channels (RyR2) contributes to the fatal arrhythmias that occur in

heart failure[9] and also induced by diabetes that these changes were attenuated with insulin treatment[10].

Liraglutide (LIRA), an analogue of human glucagon-like peptide 1 (GLP-1)[11]. GLP-1 agonists are potent glucose-lowering agents but also have potentially beneficial effects on other traditional (body weight, blood pressure (BP), and LDL cholesterol) and non-traditional risk factors (low-grade inflammation and endothelial dysfunction [12]. As the American Diabetes Association (ADA) reported that GLP-1 RA has potent glucose-lowering actions and hypoglycemia compared with intensified insulin regimen [13]. Recently, LIRA is associated with the improvement of arterial stiffness, cardiac function, and functional capacity in failing post-ischemic T2DM patients[14]. However, underlying mechanisms by which LIRA exerts beneficial actions in the myocardium remain obscure. Considering the potential functions of LIRA in the cardiovascular system, we herein, hypothesized that LIRA may attenuate diabetic cardiomyopathy injury in diabetic rats via inducing both Cav3/eNOS/NO and Cav3/RyR2 signaling pathway.

Methods

Experiment animals

Sprague-Dawley (SD) rats, male, weighing 120-160 g, were provided by the Center for Laboratory Animals of Wenzhou Medical University (License No. SCXK2015-0001). These rats were housed at room temperature, which was maintained within 23-25 °C, allowed free access to food and water, and placed under a 12-hour dark/light cycle. The study protocol was approved by the Animal Research Committee of Wenzhou Medical University. All animal experiments adhered to the Care and Use of Laboratory Animal published by the US National Institutes of Health, following the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines.

Induction of Type 2 diabetes model

T2DM models were established as previously described [15, 16]. Normal group (CON, n = 16), male rats were fed with normal diet; T2DM group (n = 50), rats were fed with high - fat and high - sugar diet (67% normal diet, 20% saccharose, 10% lard, 2% cholesterol and 1% sodium cholate). After 8 weeks of feeding, rats received a single intraperitoneal injection with streptozocin (STZ) (Sigma-Aldrich Co., St. Louis, MO, USA) of 35 mg/kg. After 3 days, rats

had fasting blood glucose concentration of ≥16.7 mmol/L were considered as T2DM rats. Rats in CON group and T2DM group were received either saline or LIRA 0.2 mg/kg per day for 4 weeks[17, 18]

Determination of blood pressure and cardiac function

Blood pressure and cardiac function were determined by invasive hemodynamic evaluation methods. A micro-catheter was inserted into the right carotid artery, the arterial blood pressure was measured using a blood pressure analyzer. Mean arterial pressure (MAP) was calculated as follows: 1/3 systolic pressure + 2/3 diastolic pressure. The micro-catheter was inserted into the left ventricle via the right carotid artery to measure the left ventricular pressure (LVP). ECG and LVP were simultaneously recorded on a polygraph (RM-6200C; Chengdu, Instrument, Chengdu, China). Computer algorithms measured heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), the first derivative of left ventricular pressure (±dP/dtmax).

Histological analysis

Hearts were immediately fixed in 4% neutral buffered formalin. Tissues were collected, embedded in paraffin, and sectioned. Sections (5 μm) were cut and mounted on positively charged glass slides. For immunohistochemistry staining, deparaffinized sections were incubated with Cav3 antibodies (BD Technology). Images were taken at a final magnification of 200 × and analyzed by Image-Pro Plus.

Measurement of NO production

Nitrite, a stable metabolite of NO with a biological reagent for NO (Nanjing Jiancheng Biological Co., China). Total nitric oxide production (NOx) in plasma was determined by measuring the concentration of Nitrite.

In Situ DHE (Dihydroethidium) Staining of the Left Ventricle

As previously described[19], left ventricle ROS levels were examined by DHE staining. The frozen left ventricle sections were incubated with DHE (10 μM) for 20 min in the dark. After washing with PBS, a fluorescence microscope (Leica, Microsystems, Germany) used to visualize the fluorescence of DHE-stained signals.

Quantitative real-time RT-PCR of left ventricle

Left ventricle tissues of rats were collected in RNAlater (Ambion, Austin, TX) and extracted total RNA using the miRNeasy kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. cDNA was amplified by real-time PCR using the primers listed in Table 1. Each sample was run in triplicate in a 20 μl reaction with 250 nM forward and reverse primers and 10

μl of Advanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA). Reactions were performed in a BIO-RAD CFX96 real-time PCR system. The cycle parameters were set as follows: an initial 3 min incubation at 95 °C, followed by 40 cycles of 95°C for 10 s, 60°C for 30 s, and 72°C for 30 s. All data were normalized to Tubala (tubulin alpha 1A gene), which was demonstrated to be stable after STZ intraperitoneal injection in our pilot work.

Table 1
Gene-specific primers used for qRT-PCR

species	Genes	Sequences
Rat	RyR2	Forward:5'-GAATCAGTGAGTTACTGGGCATGG -3'
		Reverse:5'- CTGGTCTCTGAGTTCTCCAAAAGC-3'
Rat	CCL1	Forward: 5'- TGCCATGTGGCTACAGAATGT -3'
		Reverse: 5'- CTGGGGCCGATCTCTTTGTA -3'
Rat	KCNK12	Forward:5'- TCCTGTTCTTCAACCTCTTTCT -3'
		Reverse:5'-TGATACACCGAGGGCTT-3'
Rat	Cav-3	Forward:5'- CCA AGA ACA TCA ATG AGG ACA TTG TG-3'
		Reverse:5'- GTG GCA GAA GGA GAT ACA G-3'

Western blot analysis

Left ventricle samples were lysed with lysis buffer. Protein concentrations in the supernatants were determined by Bradford Protein Assay Kit (Bio-Rad, CA, USA). The proteins were separated by electrophoresis on SDS-PAGE and then transferred onto PVDF (polyvinylidene difluoride)-Plus membrane (Micron Separations). After being blocked with 5% skim milk, incubation with primary antibodies: RyR2 antibodies (1:400), Cav-3 antibodies (1:2000), overnight at 4 °C. After that, the membrane was incubated with the corresponding secondary antibodies at room temperature for 2 h. Immunoblot was visualized with ChemiDocXRS (Bio-Rad Laboratory, Hercules, CA), and analyzed with LabImage software. Cav3 antibody was from BD Technology, RyR2 antibody was from Sigma Technology.

Immunoprecipitation

Immunoprecipitation was performed as previously describe[20]. Isolated cardiomyocytes or heart tissue were homogenized in lysis buffer. A total of 500 mg extract preparations was subjected to immunoprecipitation with 2 mg Cav3 primary antibody in the presence of 20 mL protein A/G plus-agarose. After extensive PBS washes, the immuno- precipitates were denatured and subjected to analysis for RyR2 expression by Western blot as described below.

Statistical analysis

Data were presented as mean ± SEM. The results were statistically analyzed using one-way analysis of variance (ANOVA), or paired or unpaired Student's t-test. When the ANOVA results revealed a significant difference, pairwise comparisons between means were tested by the least significant difference method (LSD). All statistical tests were performed with GraphPad Prism software, version 8.0 (GraphPad Software, San Diego, CA). $P < 0.05$ was considered statistically significant

Results

1.Characterization of the rats studied

Heart weight/ body weight was significant decreased in DM group compared with the Con group (Fig.1b). Heart rate was significantly reduced in DM group compared with Con (358.34±10.68 bpm vs. 435.92±7.35 bmp, n=8, P<0.01), After 4 weeks of LIRA treatment, the heart rate was increased in DM+L group compared with DM (Fig.1c). Fig.1d showed DM group displayed higher blood glucose than Con group (20.57±2.75 mol/L vs. 4.34±0.21 mol/L), while blood glucose level in DM+L group was lower than DM group after received LIRA (10.36±1.84 mol/L). Fig.1e showed not significantly difference in mean arterial pressure of every group.

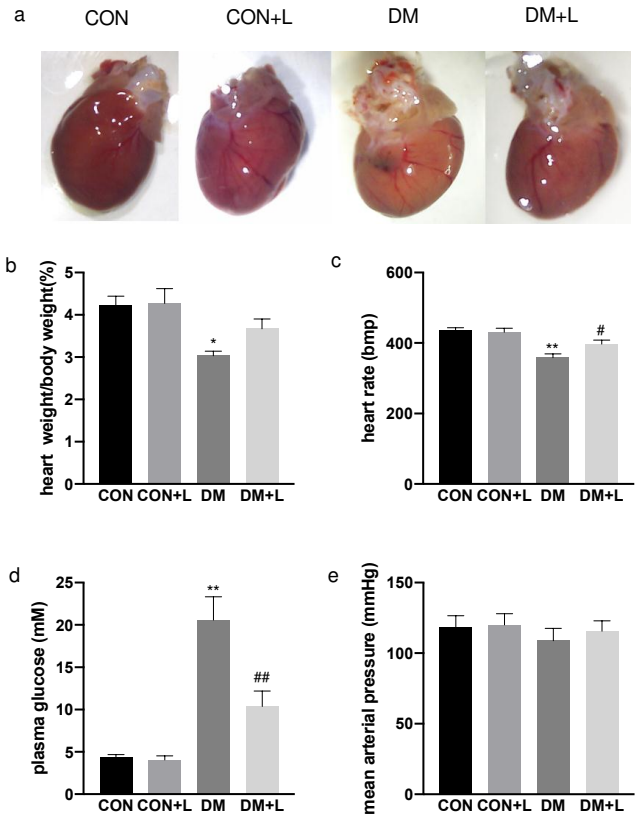

Fig 1. Control (CON) or STZ-induced diabetic (DM) rats were either untreated or treated with liraglutide (0.2mg/kg/day, DM + L) by intraperitoneal injection for 4 weeks. a. image of whole heart, b. heart weight/ body weight, c. heart rate, d. plasma glucose, e. Mean arterial pressure. All the results are expressed as Mean ± SEM, n = 8. **P < 0.01 vs. CON, ## P < 0.01 vs. DM, *P < 0.05 vs. CON, # P < 0.05 vs. DM.

2. LIRA improved cardiac function in diabetic rats

Diastolic and systolic functions of left ventricle were evaluated by measuring LVSP, LVEDP and calculating ±dP/dtmax. LVSP (91.39±4.98 mmHg), LV +dp/dtmax (4040.74±197.72 mmHg/s) were significantly reduced in DM group, and diabetic rats also exhibited reduced -dp/dtmax (2926.5±142.3 mmHg/s) and elevated LVEDP (10.87±0.83 mmHg). LIRA treatment showed a trend to enhance LVSP (110.76±5.61 mmHg) and +dP/dtmax (5860.41±200.32 mmHg), - dP/dtmax (3996.8±179.3 mmHg), decreased LVEDP (7.23±0.58 mmHg). Hemodynamic data supported LIRA improved cardiac systolic and diastolic function of diabetic rats.

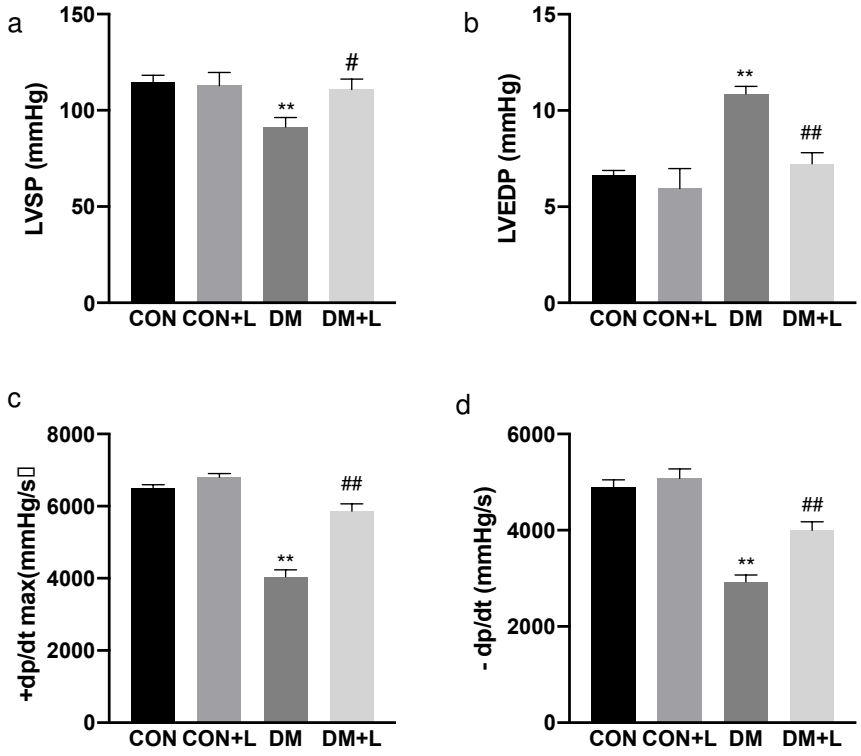

Fig 2. LIRA improves rat cardiac function. Control (CON) or STZ-induced diabetic (DM) rats were either untreated (CON+L) or treated with liraglutide (DM + L). a. LVSP, left ventricular systolic pressure; b. LVEDP, left ventricular end diastolic pressure; c, d. LV dP /dt $_{max}$, the instantaneous first derivation of left ventricle pressure. All the results are expressed as Mean ± SEM, n = 8. **P < 0.01 vs. CON, ## P < 0.01 vs. DM, # P < 0.05 vs. DM.

3.LIRA reduced ROS level in cardiac tissues of diabetic rats

Hyperglycemia-induced oxidative stress plays a critical role in the pathogenesis of cardiomyopathy [21, 22]. ROS in cardiac tissues in DM group increased significantly compared to the Con group. Treatment with LIRA counteracted this decrease (Fig.3a, b).

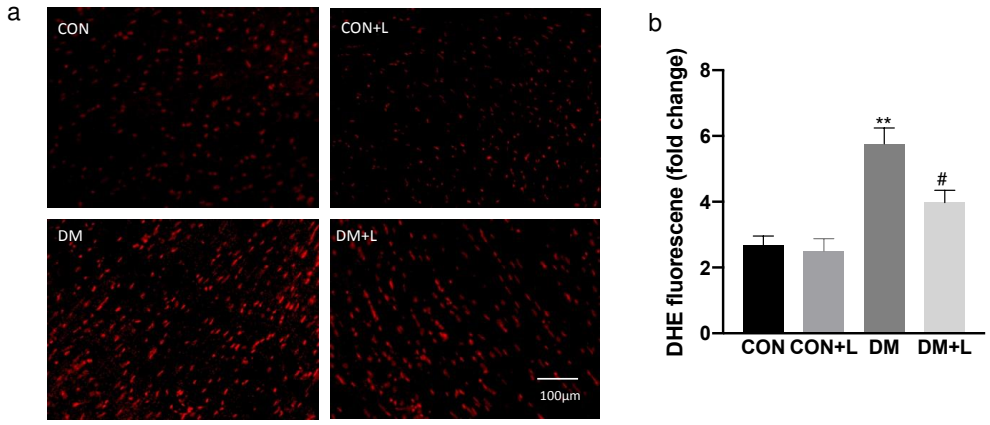

Fig.3 LIRA prevented diabetic myocardium oxidative stress. a. Images showing the levels of superoxide anions detected by DHE staining. b. Relative fluorescence density of DHE staining. Scale bar = 100 μm. All the results are expressed as Mean ± SEM, n = 4. **P < 0.01 vs. CON, $^{\#}$P<0.05 vs. DM.

4. LIRA increased Cav3 and protected against diabetes-induced LV hypertrophy

We detected Cav3 expression in heart tissues from every group rat. The expression of Cav3 was significantly decreased in the myocardium of diabetic rats (Fig.4a, b). LIRA increase Cav3 expression. Diabetic myocardial injury was significantly increased, and these were associated with hypertrophy of cardiomyocytes as reflected by an increase in cross-sectional area of cardiomyocytes (Fig. 4a, c), LIRA decreased the cross-section area of cardiomyocytes.

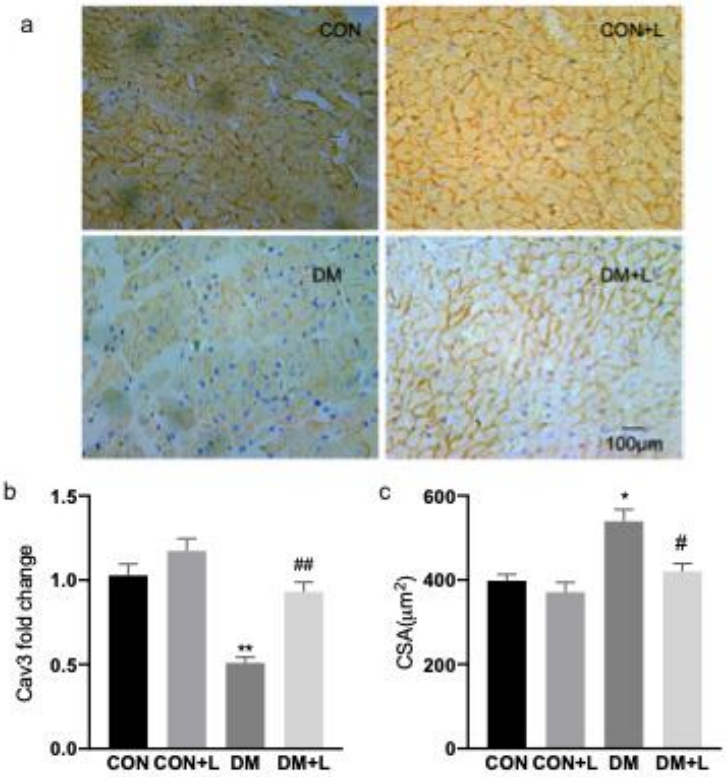

Fig. 4. LIRA treatment prevented diabetes-induced cardiac hypertrophy and increased Cav3 expression. a. Immunohistochemistry of cardiac tissues with the magnification of 400×; b. Immunohistochemistry showed Cav3-positive cells in cardiomyocytes (brown); c. cross sectional area of 4 groups. All the results are expressed as Mean ± SEM, n = 4. **P < 0.01 vs. CON, $^{\#\#}$P<0.01 vs. DM, *P < 0.05 vs. CON, $^{\#}$ P < 0.05 vs. DM.

5. LIRA improve expression of Cav3/eNOS/NO and suppress hyperphosphorylation of p-RyR2 in diabetic cardiac tissues

As shown in Fig.5 protein of Cav3 and eNOS were reduced, phosphorylations of eNOS were decreased in diabetic myocardial compared with CON group. Plasma NO level was reduced in DM group. LIRA improved expression of Cav3, eNOS in diabetic myocardial, increased phosphorylations of eNOS in both nondiabetic and diabetic rats (Fig.5c), increased NO in the plasma of diabetic rats. In diabetic cardiac tissues, RyR2 expression was reduced and phosphorylation of Ser2814 at RyR2 was increased (Fig.5e,5f). LIRA reversed the expression of RyR2 and the hyperphosphorylation of Ser2814 at RyR2 in diabetic cardiac tissues. This reversal contributed to improved cardiac systolic and diastolic function of diabetic rats.

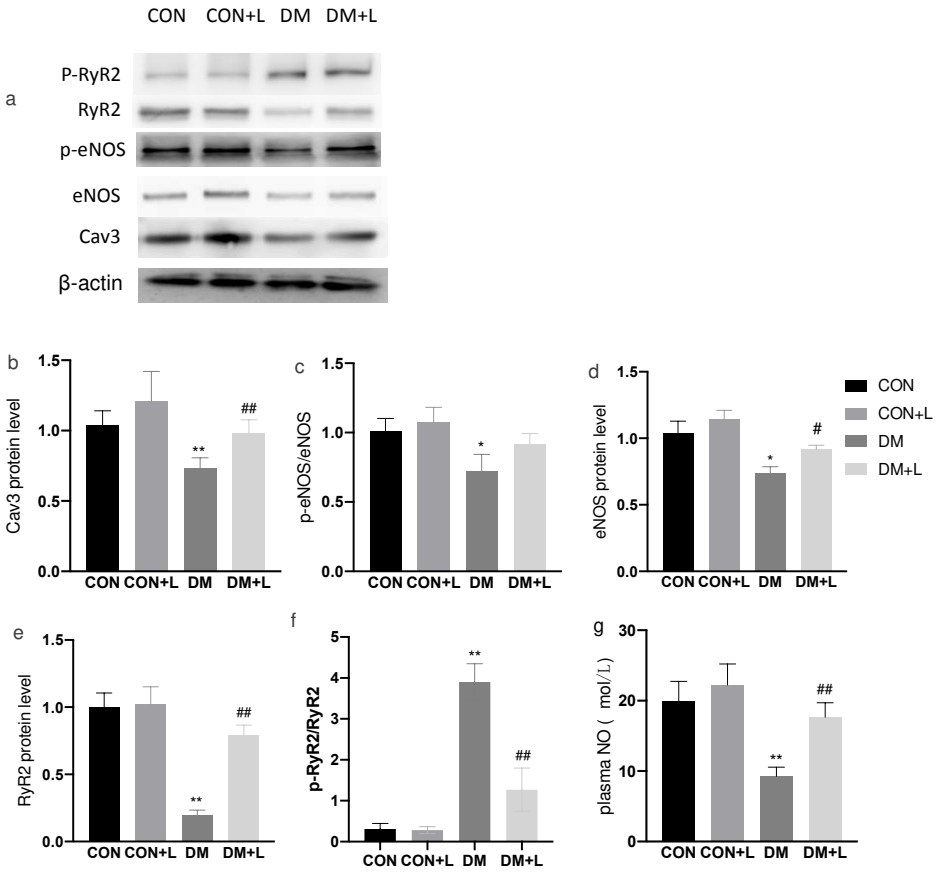

Fig.5 LIRA improve expression of Cav3/eNOS/NO signaling and suppress hyperphosphorylation of p-RyR2 in diabetic cardiac tissues. a. Western blot showing the protein expressions in cardiac tissues. b,c,d,e,f bar graph showing the relative quantification of Cav3, p-eNOS/eNOS, eNOS, RyR2, p-RyR2/RyR2. g, Concentration of NO in plasma. n=3 rats per group, all the results are expressed as Mean ± SEM. **P < 0.01 vs. CON, $^{\#\#}$P<0.01 vs. DM, *P < 0.05 vs. CON, $^{\#}$ P < 0.05 vs. DM.

6. Cav3, RyR2, CCL1 decreased in diabetic cardiac tissues

Increasing expression of Cav3/eNOS/NO partly explained that LIRA improved cardiac diastolic function of diabetic rats. To further investigate whether LIRA can increase the contractility of the heart muscle, we tested whether the downregulation of Cav3 expression affects RyR2, CCL1 and KCNK12 in diabetic cardiac tissues. Fig.6 showed mRNA of RyR2 and CCL1 were decreased in diabetic cardiac tissues.

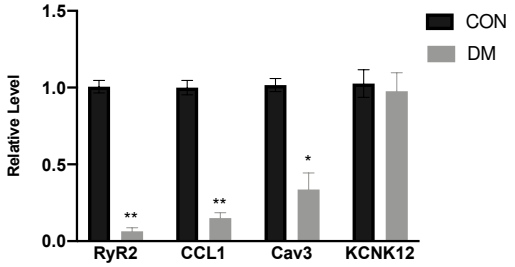

Fig. 6. Qt-PCR RyR2, CCL1 and Cav3 decreased in cardiac tissues of diabetic rats. n=3 rats per group. Data is presented as the Means ± SEM. **P<0.01 vs. CON. Cav3, caveolin-3; RyR2, Ryanodine receptor-2; CCL1, Chloride voltage-gated channel 1; KCNK12, Potassium two pore domain channel subfamily K member 12. **P<0.01 vs. CON, *P<0.05 vs. CON.

7. Interaction of Cav3 and RyR2

A lower level of RyR2 expression was observed in diabetic cardiac tissues (Fig.5e). We further tested a potential interaction between Cav3 and RyR2, CCL1 in each group. Co-immunoprecipitation was performed in the cardiac tissues. As shown in Fig.7a,7b, complex formation that occurred between Cav3 and RyR2 was enhanced in cardiac tissues of diabetic rats after LIRA treatment. While complex formation between Cav3 and CCL1 was not change in cardiac tissues of nondiabetic rats and diabetic rats. These results indicated that LIRA increased the interaction between Cav3 and RyR2.

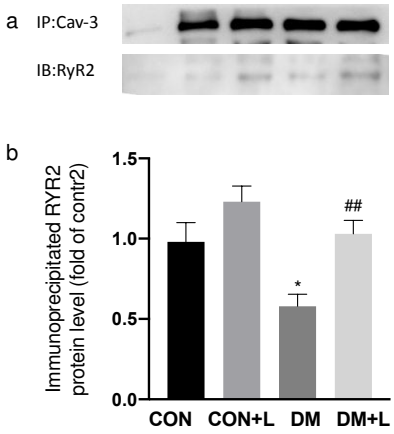

Fig. 7. LIRA enhanced RyR2 and Cav3 binding in cardiac tissues. a,b Co-immunoprecipitation of RyR2 with Cav3 in cardiac tissues. Protein lysates of cardiac tissues were incubated with anti-cav-3 antibody and protein A resin, and the immunoprecipitated proteins were blotted by anti-RyR2 antibody by Western blotting. All the results are expressed as Mean ± SEM, n = 4. *P < 0.05 vs. CON, $^{\#\#}$P<0.01 vs. DM.

Discussion

The present study demonstrates that LIRA attenuates diabetic cardiomyopathy injury in diabetic rats possibly through improving Cav3/eNOS/NO signaling and increasing interaction of Cav3 and RyR2. Expression and phosphorylation of eNOS decreased in cardiomyocytes of diabetic rats. Cardiac-specific down expression of Cav3 in diabetic rats decreased expression of RyR2 while hyperphosphorylated at Ser2814 of RyR2. LIRA reversed the expression of eNOS and RyR2. This reversal possibly contributed to improved cardiac systolic and diastolic function of diabetic rats.

Type 2 diabetes is a complex metabolic disorder that is characterized by hyperglycemia and associated with a high risk of cardiovascular, microvascular, and other complications[23]. Regulatory authorities have mandated cardiovascular safety assessments of new diabetes treatments[24]. Our findings may have implications for clinical trial results using GLP-1 agonists to treat DM. Trials have found that these newer glucose-lowering drugs significantly reduce the incidence of major cardiovascular events in diabetic patients and cardiovascular benefits are unrelated to their glycemic control effects [25, 26]. Cardiac diastolic dysfunction and arterial stiffness are early manifestations of obesity-associated prediabetes[27, 28]. Both left ventricle diastolic and systolic dysfunctions of the myocardium in DM rats were observed in our work. Recently LEADER (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results) trial has reported the cardiovascular benefits of LIRA. LIRA significantly reduced the risk of the 3 major adverse cardiovascular events (death from cardiovascular causes, nonfatal stroke, and nonfatal myocardial infarction) in patients with DM who were at high risk for cardiovascular events[29]. Furthermore, LIRA is associated with improvement of cardiac function and functional capacity in failing post-ischemic type-2 diabetes mellitus patients[30], though the mechanism of protective effect remained incompletely understood. In the present study， we demonstrate LIRA effect on glucose reduction, do not affect blood pressure but attenuated increased left ventricular (LV) minimum pressure and ameliorated LV systolic and diastolic dysfunction in DM rats in comparison with the vehicle. Oxidative stress is the main mechanism of impaired cardiac function in DM patients [29]. Reactive oxygen species (ROS) are usually produced in massive amounts via glucose and lipid peroxidation, and this leads to diabetic complications[31]. Recent studies have shown that ROS formation is exacerbated in diabetics due to a glycolytic metabolic shift [34]. Hyperglycemia and insulin resistance results in excess ROS production [35]. RyR2 is the major SR Ca^{2+} release channel and an important oxidative target in cardiomyocytes[32]. Several Bilayer studies have shown that RyR2 channel activity is increased in the presence of ROS, whereas reducing agents decrease the RyR2 activity[33]. RyR hyperphosphorylation can lead to mitochondrial Ca^{2+} overload, thereby facilitating ROS production[34]. Liraglutide treatment decreased apoptosis and intracellular ROS in H9C2 cardiomyocytes in high glucose[35].We found LIRA lowered superoxide anions excessive production in diabetic myocardium which implicated that oxidative stress damage in the DM heart was increased and reversed after treatment with LIRA.

Cav3 has been reported to be related to many cardiovascular diseases. Cav3 overexpression exerts a protective effect on diabetic hearts against ischemia/reperfusion damage through the β_2AR, cAMP/PKA, and BDNF/TrkB signaling pathways[36]. Cav3 expressions are required for isoflurane-induced cardiac protection from hypoxia and ischemia/reperfusion injury[37]. However, in diabetes, the cardiac Cav3 expression is impaired by hyperglycemia-induced oxidative stress[38]. LIRA is associated with improvement of cardiac function and functional capacity in failing postischemic type 2 diabetes mellitus patients[33]. Although many studies have demonstrated the protective role of Cav3 in multiple cardiac diseases, few studies have focused on the myocardial protective mechanism of LIRA in type 2 diabetic rats is through Cav3. Recent studies have showen that myocardial eNOS protein level was decreased in DM rats[39]. Caveolae have long been associated with eNOS[40], which produces NO. LIRA treatment restored insulin - mediated eNOS activation in endothelial cells freshly isolated from patients with DM [36]. We found that Cav3 expression was decreased and associated with reduced eNOS protein levels and NO production in DM rats. These abnormalities were accompanied by reduced heart weight/body weight ratio and heart rate, resulting in an increased left ventricular end-diastolic pressure. Our data showed that LIRA improved cardiac function by increasing Cav3 level and enhancing eNOS activity and NO production.

Early-onset diastolic dysfunction and late-onset systolic dysfunction have been associated with both T1DM and T2DM, in which alteration in Ca^{2+} signaling is major important [38]. The high-conductance intracellular Ca^{2+} channel RyR2 is essential for the coupling of excitation and contraction in cardiac muscle[41]. Accordingly, increasing the magnitude of calcium flux through RyR2 is a critical element in increasing the force of contraction and consequently the amount of blood ejected from the heart per beat[42]. Phosphorylation of cardiac RyRs is an important modulatory mechanism of Sarcoplasmic reticulum(SR) Ca^{2+} release characteristics[43]. Therefore, we tested the RyR2 and the extent of RyR2 phosphorylation. We found that DM promoted the phosphorylation of Ser2814 at RyR2 but decreased the RyR2 expression. LIRA reversed the expression of RyR2 and the hyperphosphorylation of Ser2814 at RyR2 in diabetic cardiac tissues. A small number of RyR clusters were in junctional couplings between subsarcolemmal SR and caveolae, a relatively small fraction Cav3 colocalized with RyR clusters in the t-system although Cav3 was expressed widely in the t-system[44]. The positioning of Cav3 adjacent to isolated RyR in the cell interior is a characteristic of other mammalian cardiomyocytes[33]. To further explore the role of Cav3 in regulating RyR2 phosphorylation, co-immunoprecipitation was performed in the cardiac tissues. The data indicated that LIRA increased the interaction between Cav3 and RyR2, which may increase the myocardial contraction ability of diabetic rats.

Conclusions

In summary, the present study demonstrates that myocardial dysfunction is associated with cardiac-specific down expression of Cav3, expression and phosphorylation of eNOS decreased and hyperphosphorylated at Ser2814 of RyR2 in cardiomyocytes of diabetic rats. Our data

suggest that the GLP-1 RA Liraglutide attenuates diabetic cardiomyopathy injury by reducing ROS level, improving Cav3/eNOS/NO signaling and increasing interaction of Cav3 and RyR2 in cardiac tissues. It contributed to improved cardiac systolic and diastolic function of diabetic rats. Therefore, these results will provide research strategies for more internal details of clinical trials involving liraglutide in the treatment of diabetes, including cardiovascular protection.

Abbreviations

LIRA：Liraglutide；GLP-1：Glucagon-like peptide 1; DM:Diabetes mellitus; T2DM: Type 2 diabetes mellitus; LVSP: Left ventricular systolic pressure ; LVEDP: Left ventricular end diastolic pressure; Cav3: Caveolin-3; RyR2: Ryanodine receptor-2; eNOS: Endothelial nitric oxide synthase; NO: Nitric Oxide; ROS: Reactive oxygen species; CCL1: Chloride voltage-gated channel 1; KCNK12: Potassium two pore domain channel subfamily K member 12.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Animal Research Committee of Wenzhou Medical University (License No. SCXK2015-0001).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

Not applicable

Authors' contributions

XL performed experiments and wrote the manuscript; ZBN performed experiments; JWW, XYY performed T2DM models; YJS Co-immunoprecipitation; RHL In situ DHE staining; WP Measured NO production; HC supervisor and planning of study; WJW supervisor, planning of study, reviewed/revised manuscript. All authors read and approved the final manuscript.

Founding

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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