Liraglutide ameliorates diabetic cardiomyopathy via activating Cav3/eNOS/NO signaling and enhancing interaction of Cav3 with RyR2

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Research Article

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

Posted Date: May 23rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1647971/v1

Abstract

Purpose

Liraglutide (LIRA), a Glucagon-like peptide-1 receptor agonist (GLP-1RA), showed potent cardioprotective effects of diabetic cardiomyopathy (DCM) with the mechanism remained incompletely understood.

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. Cultured H9C2 cells with small interfering RNA (siRNA) of Cav3 under high glucose (HG), western blot was performed to understand the molecular mechanism between Cav3 and RyR2 with LIRA.

Results

Based on our results, 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. LIRA improved cardiac systolic and diastolic function, attenuate diabetic cardiomyopathy injury by improving Cav3/eNOS/NO signaling and increasing interaction of Cav3 and ryanodine receptor 2 (RyR2) in diabetic cardiac tissues.

Conclusion

In summary, we found that Liraglutide ameliorates cardiac dysfunction in rats with type 2 diabetes mellitus via improving Cav3/eNOS/NO signaling and increasing interaction of Cav3 and RyR2.

Introduction

Diabetes has become the most common metabolic disorder in the world. Diabetic cardiomyopathy (DCM) is a major cause of morbidity and mortality in diabetic patients[1, 2]. DCM is characterized in its early stages by diastolic relaxation abnormalities and later by clinical heart failure that occurs independent of dyslipidemias, hypertension, and coronary artery disease[3]. DCM is a common and still underdiagnosed finding in patients with diabetes that may have a significant impact on quality of life. Hence, the discovery of new pharmacological targets or the development of novel therapeutic strategies to treat DCM is paramount.

Caveolae are a specialized type of lipid raft that is stabilized by oligomers of the caveolin protein. Cav3, the dominant isoform of cardiomyocyte caveolae, is reduced in diabetic hearts in which oxidative stress is increased[4]. Within these microdomains, caveolins interact with numerous signaling molecules such as endothelial nitric oxide synthase(eNOS), PI3K, and MER/MRK etc [5]. Accumulating evidence has revealed that cell apoptosis impaired eNOS/NO contribute to diabetes-induced myocardial injury [6, 7]. Dysfunctional calcium release channels (RyR2) induced by diabetes that these changes were attenuated with insulin treatment[8]. However, it is still unknown whether the relationship between Cav3 and RyR2 will affect diabetic cardiomyopathy.

Liraglutide (LIRA), an analogue of human glucagon-like peptide 1 (GLP-1)[9]. 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 [10]. As the American Diabetes Association (ADA) reported that GLP-1 RA has potent glucose-lowering actions and hypoglycemia compared with intensified insulin regimen [11]. Recently, LIRA is associated with the improvement of arterial stiffness, cardiac function, and functional capacity in failing post-ischemic T2DM patients[12]. 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 DCM injury in diabetic rats via inducing both Cav3/eNOS/NO and Cav3/RyR2 signaling pathway.

Material And 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.

Type 2 diabetes model and experimental group

T2DM models were established as previously described [15, 16]. After 3 days adaptation feeding, the rats were divided into 2 groups randomly: Normal group (CON, n = 16), the rats were fed with normal diet; type 2 DM group (DM, n = 50), the rats were fed with high-fat and high-sugar diet (67% normal diet, 20% saccharose, 10% lard, 2% cholesterol and 1% sodium cholate). At week 0 and 8 after feeding, fasting plasma glucose, fasting insulin were measured and the insulin sensitivity index was calculated in both groups. Then the DM group 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 were received intraperitoneal

injection with LIRA 0.2 mg/kg per day (CON+L, n=8) and DM group were also received LIRA 0.2 mg/kg per day (DM+L, n=8) for 4 weeks [17, 18]. The rats of CON and DM group received either saline.

Measure of Insulin and Calculate the Insulin Sensitivity Index

1 ml of tail vein blood was obtained after fasting for 12 h, and placed at room temperature for 30 min. The blood samples were centrifuged to obtain supernatants. Measured the insulin content of supernatants by ELISA kits (Haixi Tang Biotechnology Co., Ltd., Shanghai, China). The insulin sensitivity index was calculated according to the following formula: insulin sensitivity index = 1/ (fasting glucose × fasting insulin). Because the value was not normally distributed, the natural logarithm was used for the calculation[13].

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

Heart tissues 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.

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'
Rat	Cav-3	Reverse:5'-TGATACACCGAGGGCTT-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[14]. 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.

Transfection with Small Interfering RNA in H9C2 Cells

H9C2 cells were cultured in DMEM containing 10% FBS in a humidified atmosphere (5% CO_2) at 37°C and plated in 6-well plates to form a monolayer for 24 h. For mimic the increased glucose level in diabetes, normal concentration glucose, 5.5 mM glucose (in mM) (D-glucose, 5.5; mannitol, 14.5; NaCl, 81; KCl, 4.0; $CaCl_2$, 1.6; pH 7.4), high concentration glucose, 33.3 mM glucose (in mM) (D-glucose, 33.3; NaCl, 81; KCl, 4.0; $CaCl_2$, 1.6; pH 7.4; HG). The cells were bathed in Hepes-buffered solution for 24 h. Normal Hepes-buffered solution (in mM) (NaCl, 135; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 1; Hepes, 10; pH 7.4). H9C2 cells were transfected with a scrambled siRNA or Cav-3 siRNA at a final concentration of 80 nM (100 nM; Santa Cruz Biotechnology, Santa Cruz, CA) for 6 h by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols[15]. Cells were used for treatments LIRA 100 nM after 48 h of incubation[16].

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

Validation of type 2 diabetic rat models

We had the model of type 2 diabetic rats after a high-sugar-fat diet for 8 weeks with a single 35 mg/kg administration of STZ. 3 days after STZ injection, blood glucose levels in the DM group were dramatically elevated compared with those of the CON group (24.50±2.37 mol/L vs. 5.03±0.79 mol/L, $P < 0.01$, Table 4). The plasma insulin levels increased significantly ($P < 0.01$, Table 2), while insulin sensitivity index ISI decreased in the DM group compared with the CON group ($P < 0.01$, Table 3). DM+L group displayed higher ISI (5.78±0.24, $P < 0.01$) and lower blood glucose (10.36±1.84 mmol/L, $P < 0.01$) than the DM group after receiving LIRA for 4 weeks.

Table 2 Insulin level in CON and DM group of rats (Mean ± SEM)

Group	insulin level(mIU/L)		
	0 week	8 week	After LIRA 4week
CON	15.2±1.61	16.4±1.12	15.7±2.36
DM	14.8±1.84	38.9±2.80**	31.6±2.05**

**P < 0.01 vs. DM 0 week, n = 8

Table 3 Insulin sensitivity index in CON and DM group of rats (Mean ± SEM)

Group	ISI		
	0 week	8 week	After LIRA 4week
CON	4.19±0.32	3.06±0.17	4.42±0.21
DM	4.31±0.23	3.062±0.19**	5.78±0.24##

**P < 0.01 vs. DM 0 week, ## P < 0.01 vs. DM after DM 8 week, n = 8

Table 4 Blood glucose in CON and DM group of rats (Mean ± SEM)

Group	Blood glucose(mmol/L			
	0 week	8 week	After STZ 3d	After LIRA 4week
CON	4.34±0.34	5.1±0.9	4.48±0.62	5.3±1.04
DM	5.03±0.79	5.13±0.68	24.5±2.37**	10.36±1.84**##

**P < 0.01 vs. DM 0 week, ## P < 0.01 vs. DM after STZ 3d, n = 8

LIRA improved cardiac function in diabetic rats

Heart weight/ body weight and heart rate were significantly decreased in the DM group compared with the CON group (Fig.1e). The heart rate was increased in the DM+L group compared with the DM group (Fig.1f) after 4 weeks of LIRA treatment. The mean arterial pressure of every group was not significantly showed the difference (Fig.1g). Diastolic and systolic functions of the left ventricle were evaluated by measuring LVSP, LVEDP, and calculating ±dP/dt max (Fig.1 a-d). LVSP (91.39±4.98 mmHg), LV +dp/dt max (4040.74±197.72 mmHg/s) were significantly reduced in DM group, and diabetic rats also exhibited reduced -dp/dt max (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/dt max (5860.41±200.32 mmHg), -dP/dt max (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 1. LIRA improves rat cardiac function. 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. e. heart weight/ body weight, f. heart rate, g. 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.

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

We detected Cav3 expression in heart tissues from every group of rats. The expression of Cav3 was significantly decreased in the myocardium of diabetic rats, LIRA increased Cav3 expression (Fig. 2a, b). 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. 2a, c), LIRA decreased the cross-section area of cardiomyocytes.

As shown in Fig. 2e, protein expression of Cav3 and eNOS were reduced, phosphorylations of eNOS decreased in diabetic myocardial compared with the CON group. LIRA improved expression of Cav3, eNOS in diabetic myocardial, increased phosphorylations of eNOS in both nondiabetic and diabetic rats (Fig. 2e, f, g, i). The plasma NO decreased in diabetic rats and increased after LIRA treatment (Fig.2d). These may help to improve myocardial diastolic function. In diabetic cardiac tissues, expression of RyR2 was reduced and phosphorylation of Ser2814 at RyR2 increased (Fig. 2h, j). LIRA reversed the expression of RyR2 and the hyperphosphorylation of Ser2814 at RyR2 in the DM+L group. This reversal contributed to improved cardiac systolic and diastolic function of diabetic rats.

Fig. 2 LIRA treatment prevented diabetes-induced cardiac hypertrophy, improved expression of Cav3/eNOS/NO signaling and suppressed hyperphosphorylation of RyR2 in diabetic cardiac tissues. a. Immunohistochemistry of cardiac tissues with the magnification of 200×; showed Cav3-positive cells in cardiomyocytes (brown); b. bar graph showing Cav3 expression on cardiac tissue; c. cross sectional area of 4 groups; d. plasma NO concentration; e, Western blot showing the protein expressions in cardiac tissues; f-j bar graph showing the relative quantification of, p-eNOS/eNOS, Cav3, RyR2, eNOS, p-RyR2/RyR2. 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.

Interaction of Cav3 and RyR2 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. mRNA of RyR2 and CCL1 were decreased in diabetic cardiac tissues (Fig. 3 a). A lower level of RyR2 expression was observed in diabetic cardiac tissues (Fig.2 h). We further tested a potential interaction between Cav3 and RyR2, CCL1 in each group. Co-immunoprecipitation was performed in the cardiac tissues. Complex formation that occurred between Cav3 and RyR2 was enhanced in cardiac tissues of diabetic rats after LIRA treatment (Fig.3 b,c). 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.

We further tested whether downregulation of Cav3 expression affected RyR2 in H9C2 with high glucose using siRNA. Expression of Cav3 and RyR2 decreased, phosphorylation of RyR2 increased in H9C2 with high glucose. After transfected with Cav3 siRNA, as expecting the expression of Cav3 decreased, expression of RyR2 downregulated either, p-RyR2/RyR2 was enhanced. LIRA upregulated the expression

of Cav3 (Fig.3 d,f) and RyR2(Fig.3 d,e), and downregulated phosphorylation of RyR2 in the HG+L group (Fig.3 d, g). The result showed LIRA changed the interaction of Cav3 and RyR2.

Discussion

The present study demonstrates that LIRA attenuates DCM injury in diabetic rats possibly through improving Cav3/eNOS/NO signaling and increasing interaction of Cav3 and RyR2. Cardiac-specific down expression of Cav3 decreases expression of RyR2 and hyperphosphorylated at Ser2814 of RyR2 in diabetic rats. LIRA upregulates the expression of Cav3/RyR2 and downregulates phosphorylation of RyR2. This reversal result of LIRA 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[17]. type 2 diabetes mellitus can promote the development of a specific form of cardiomyopathy that is independent of coronary artery disease and hypertension. Cardiac diastolic dysfunction and arterial stiffness are early manifestations of obesity-associated prediabetes[18, 19]. Both left ventricle diastolic and systolic dysfunctions of the myocardium in DM rats were observed in our work. Regulatory authorities have mandated cardiovascular safety assessments of new diabetes treatments[20]. 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 [21, 22]. Our findings may have implications for clinical trial results using GLP-1 agonists to treat DM. 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[23]. Furthermore, LIRA is associated with improvement of cardiac function and functional capacity in failing post-ischemic type-2 diabetes mellitus patients[24], 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 minimum pressure and ameliorated left ventricular systolic and diastolic dysfunction in DM rats in comparison with the vehicle.

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[25]. Cav3 expressions are required for isoflurane-induced cardiac protection from hypoxia and ischemia/reperfusion injury[26]. However, in diabetes, the cardiac Cav3 expression is impaired by hyperglycemia-induced oxidative stress[27]. 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 shown that myocardial eNOS/NO decreased in DM rats[28].

Caveolae have long been associated with eNOS[29], 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[30]. 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[31]. Phosphorylation of cardiac RyRs is an important modulatory mechanism of Sarcoplasmic reticulum(SR) Ca^{2+} release characteristics[32]. 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[33]. The positioning of Cav3 adjacent to isolated RyR in the cell interior is a characteristic of other mammalian cardiomyocytes[34]. LIRA can protect against diabetic cardiomyopathy by inactivating the ER stress pathway[16]. 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.

Conclusion

In summary, the present study demonstrates that DCM 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 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. These results will provide research strategies for the utility of GLP-1 agonists 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; CCL1: Chloride voltage-gated channel 1; KCNK12: Potassium two pore domain channel subfamily K member 12.

Declarations

Funding

This work was supported by grants from Natural Science Youth Project Foundation of Zhejiang Province (LQ17H020004) Service programs of technology innovation of Wenzhou Association for Science and Technology (kjfw20). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interest statement

XL, ZBN, JWW, XYY, YJS, RHL, WP, HC, WJWs declare that they have no relevant financial or non-financial interests to disclose

Author 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.

a data availability statement

Data openly available in a public repository that issues datasets with DOIs

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Figures

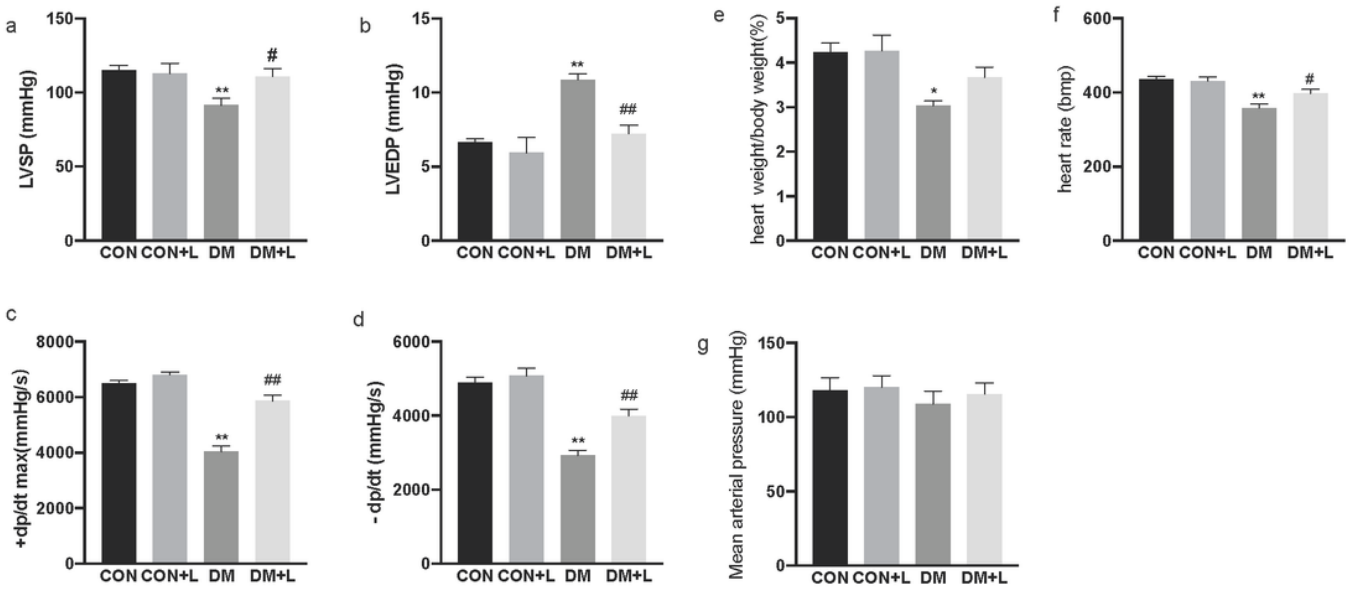

Fig 1. LIRA improves rat cardiac function. a. LVSP, left ventricular systolic pressure; b. LVEDP, left ventricular end diastolic pressure; c,d. LV dP /dt $_{max}$, the instantaneous first derivation of left ventricle pressure. e. heart weight/ body weight, f. heart rate, g. Mean arterial pressure. All 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.

Figure 1

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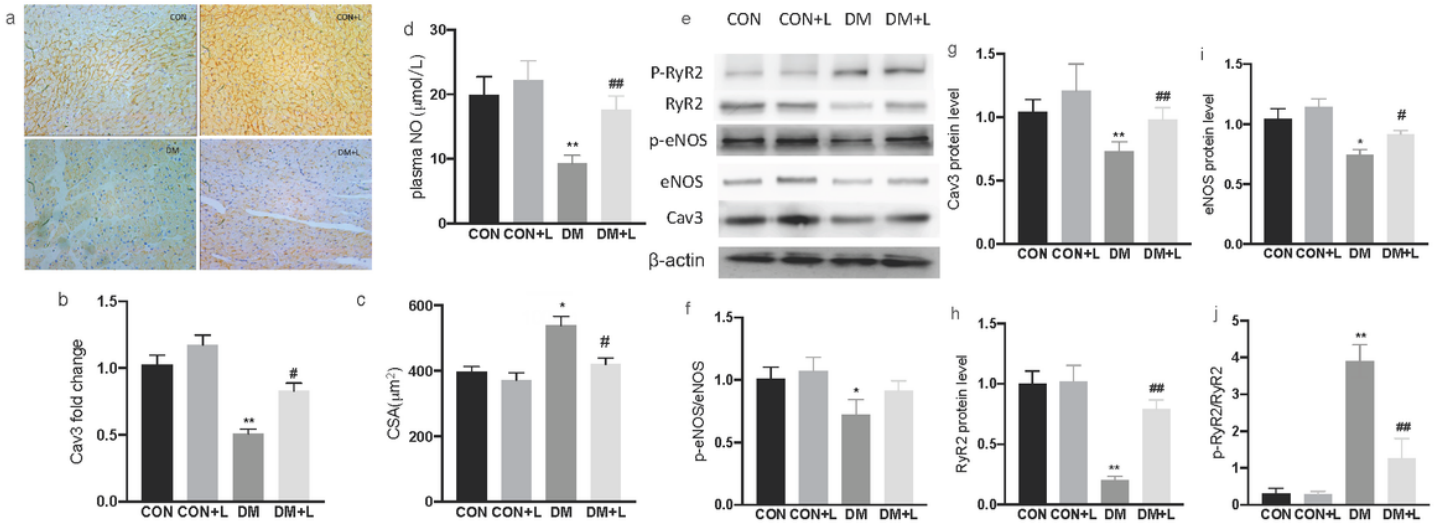

Fig. 2. LIRA treatment prevented diabetes-induced cardiac hypertrophy, improved expression of Cav3/eNOS/NO signaling and suppressed hyperphosphorylation of RyR2 in diabetic cardiac tissues. a. Immunohistochemistry of cardiac tissues with the magnification of 200×; showed Cav3-positive cells in cardiomyocytes (brown); b. bar graph showing Cav3 expression on cardiac tissue; c. cross sectional area of 4 groups; d. plasma NO concentration; e, Western blot showing the protein expressions in cardiac tissues; f-j bar graph showing the relative quantification of, p-eNOS/eNOS, Cav3, RyR2, eNOS, p-RyR2/RyR2. 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.

Figure 2

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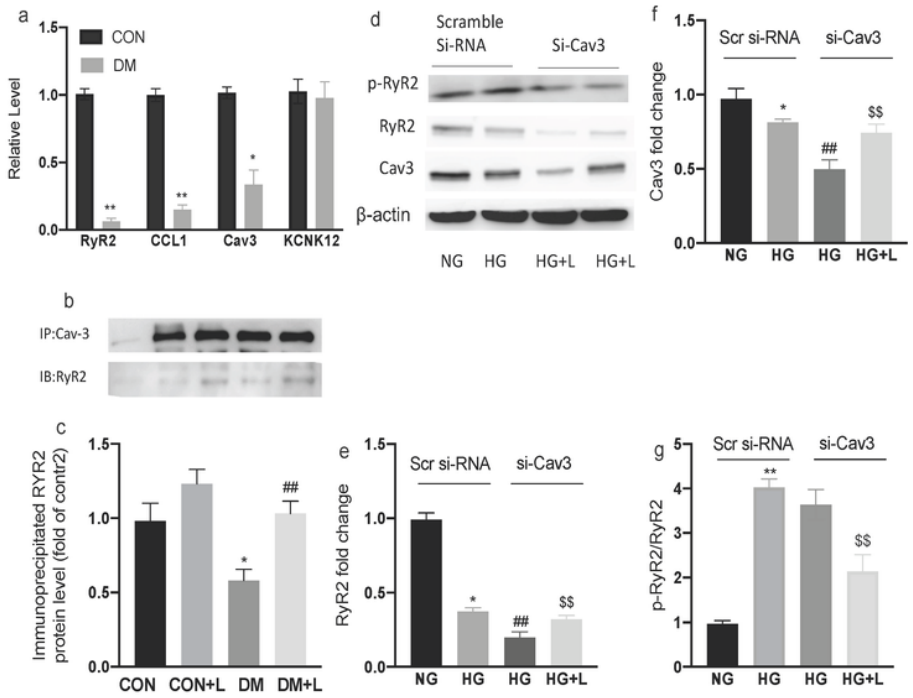

Fig. 3. LIRA improve diabetic cardiac function that was associated with increased the interaction between Cav3 and RyR2. a. Relative mRNA level of RyR2, CCL1 and Cav3 in cardiac tissues measured by quantitative real-time PCR of CON and DM; b, c Co-immunoprecipitation of RyR2 with Cav3 in cardiac tissues. 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. d,e,f,g Protein expression of RyR2, Cav-3 and phosphorylation of RyR2 in H9C2 by transfected with Cav-3-siRNA or scrambled siRNA in different medium. NG: normal glucose, HG: high glucose, HG+L: high glucose + LIRA. All the results are expressed as Mean ± SEM, n = 4. **P<0.01vs. CON, $^{\#\#}$P<0.01 vs. DM, *P < 0.05 vs. CON, $^{\$\$}$ P < 0.01 vs. HG.

Figure 3

See image above for figure legend