Elucidation of The Effects and Underlying Mechanism of Aerobic Interval Training Combined With Liraglutide On Diabetic Cardiomyopathy

Huan Cai
Tianjin University of Sport
Linling Zhou
Graduate School of Hebei Medical University
Jingqin Liu
Tianjin University of Sport
Zelin Li
Hebei General Hospital
Shuchun Chen (chenshuc2014@163.com)
Hebei General Hospital

Research Article

Keywords: diabetic cardiomyopathies, liraglutide, aerobic interval training, joint intervention, cardioprotection

Posted Date: January 3rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1150327/v1

Abstract

*objective: T*his study intended to explore the hypoglycemic and cardioprotective effects of 8-week aerobic interval training combined with liraglutide and elucidate the underlying mechanisms.

Method: Male Wistar rats were randomly divided into 5 groups - normal control (CON), diabetic cardiomyopathy (DCM), high-dose liraglutide (DH), low-dose liraglutide DL , and aerobic interval training combined with liraglutide (DLE). The cardiac function of rats ,the FBG the levels of fasting insulin (FIN), $HbA_{1c,}$ the total collagen content , AGEs, the mRNA expression of myocardial remodeling genes *BNP*, *GSK3β*, *α-MHC*, and *β-MHC* ,the expression of GLP-1 and GLP-1R proteins, Insulin resistance (HOMA-IR) and beta-cell function (HOMA-β) was analyze.

Results: During the intervention, the FBG in each intervention group significantly decreased compared to the DCM group. After 8 weeks,the DH, DL, and DLE groups showed improved blood glucose-related indices and cleared the accumulated AGEs in the DCM groups. The heart function in the DLE groups was significantly improved than that in the DH and DL groups. The relative expression of *BNP* mRNA in the DH, DL, and DLE groups significantly reduced compared to the CON and the DCM group .Compared to the DCM group,the relative expression of *α-MHC* mRNA increased significantly and *β-MHC* mRNA decreased notably in the myocardium of the DH, DL, and the DLE group.The expression of GLP-1 in the myocardial tissue of rats in the DH group was higher than that in the DL and DLE groups. GLP-1R expression in the myocardial tissue in the DLE group was higher than that in the DH , DL and the DCM groups .

Conclusion: Liraglutide combined with AIT intervention significantly reduced FBG and the fluctuations in FBG, alleviated myocardial fibrosis, improved cardiac function in DCM rats, supporting the efficacy of the combined pharmaceutical and physical intervention, and reduced the cost of treatment.

Introduction

Diabetic cardiomyopathy is a pathophysiological state induced by diabetes mellitus (DM) in which heart failure occurs in the absence of coronary artery disease, hypertension, and valvular heart disease [2]. Liraglutide stimulates insulin secretion, inhibits the secretion of glucagon, and suppresses appetite to reduce blood sugar levels [3]. Several guidelines in the United States and Europe have listed GLP-1 receptor agonists as second-line drugs for the treatment of diabetes [4, 5]. The guidelines for the prevention and control of type 2 diabetes in China (2020 Edition) [6] are recommended that regardless of whether HbA_{1c} is within normal limits, type 2 diabetes patients with atherosclerotic cardiovascular disease or high cardiovascular risk should use GLP-1 receptor agonists .However, the treatment of diabetes is costly and may be a burden for the patients.

Exercise is one of the "five carriages" for the treatment of diabetes. It increases insulin sensitivity and decreases blood sugar, lipids, and cardiovascular risk factors. Aerobic exercises combined with metformin therapy improved cardiopulmonary function and the quality of life in patients with insulin resistance[7].Rosiglitazone and aerobic exercise significantly improved insulin sensitivity in obese rats

individually, and their combined treatment restores insulin sensitivity to normal levels in diabetic patients [8]. However, there are no reports on the mechanism underlying the hypoglycemic effect of liraglutide combined with exercise. In aerobic interval training (AIT),the oxygen debt and consumption can be used to estimate the ratio of aerobic to anaerobic energy supply and improve the body's aerobic capacity and the maximum rate of oxygen consumption (VO_2 max). Therefore, AIT is more suitable for the rehabilitation of cardiovascular patients. However, the effect of AIT combined with liraglutide on the heart functioning in DCM has not been reported.

Therefore, the current study intended to explore the protective effect of 8-week aerobic interval training combined with liraglutide on the heart of DCM rats, compare whether the effect of the combined intervention is superior to drug intervention alone, and explore its underlying mechanism.

Experimental Results

Changes in FBG of rats during the 8-week intervention

At wk0, the FBG levels of the diabetic rats were not significantly different ($P > 0.05$) but were significantly higher than those in the CON group ($P < 0.01$), indicating that the diabetes model was successfully established. At wk1, the FBG levels of rats in the DH, DL, and DLE groups were significantly lower than those in the DCM group ($P < 0.05$). The FBG of the rats in the DH group decreased the most compared to the DL and DLE groups but the differences were not significant ($P > 0.05$). In wk2, the FBG levels of the rats in the DH and DL groups increased slightly but those in the DLE group continued to decrease. After wk2, FBG was monitored once every two weeks. FBG decreased to varying degrees in the groups. The FBG of rats in the DH and DL groups altered to a certain extent but those in the DLE group decreased steadily. The FBG levels in each of the intervention groups were significantly lower than those in the DCM group ($P < 0.05$). The FBG levels of rats in the CON group remained stable throughout the 8 weeks.(in Fig. 2)

Comparison of related indices of the serum metabolites across the experimental groups

After 8 weeks of intervention, the levels of FBG, HOMA-IR, and HbA_{1c} increased and HOMA-β decreased significantly in the DCM group compared to the CON group ($P < 0.01$). However, the levels of FINs were not significantly different across the groups ($P > 0.05$). Compared to the DCM group, the DH, DL, and DLE groups demonstrated a significant decrease in the levels of FBG, HOMA-IR, and HbA_{1c} ($P < 0.01$) and an increase in the HOMA-β ($P < 0.01$). The levels of FINs in the DH group were higher than those in the DCM group ($P < 0.05$). Although the levels of FINs in other groups were higher than the DCM group, the differences were not significant ($P > 0.05$).(in Table2)

Table 2
Establishment of diabetic cardiomyopathy models

	CON	DCM	DH	DL	DLE
FBG(mmol/L)	5.82±0.87	19.19±2.02**	10.37±2.62##	11.89±2.19##	10.72±2.24##
FINs(IU/L)	13.38±2.90	16.64±3.83	22.85±3.59#	20.00±4.17	20.62±2.16
HOMA-IR	3.42±0.75	14.29±4.37**	10.44±2.81	10.40±1.95	9.74±1.84
HOMA-β	138.30±80.00	21.39±4.92**	80.53±49.78	53.52±30.86	64.15±27.70
HbA1c (mmol/L)	6.35±0.52	17.27±2.75**	10.65±1.00##	11.77±1.35##	10.97±1.55##

Note 2: One-way analysis of variance was performed; **, P < 0.01 compared to the CON group. #, P < 0.05; ##, P < 0.01 compared to the DCM group.

Comparison of the heart function of rats across the groups

After 8 weeks of intervention, the results of echocardiography showed that compared to the control group, the EF, FS, and CO of the rats decreased significantly (P < 0.01), the E/A ratio increased significantly (P < 0.01), and IVRT increased but not significantly (P > 0.05) in the DCM group. Compared to the DCM group, the DH, DL, and DLE groups showed a significant decrease in the E/A ratio (P < 0.05), and a significant increase in EF (P < 0.05). The FS of the DLE group was higher than that of the DCM group (P > 0.05), and the EF was significantly higher than that of the DH and DL groups (P > 0.05). No significant changes were observed in LVEDD, LVESD, and IVRT (P > 0.05) across the groups.(in Table3)

Table 3
Comparison of cardiac function across the experimental groups

	CON	DCM	DH	DL	DLE
LVEDD(mm)	6.70±0.41	7.26±0.96	6.83±0.89	6.70±0.64	6.30±0.95
LVESD(mm)	3.86±0.58	4.42±1.20	4.32±0.85	4.09±0.50	3.56±1.15
EF(%)	78.45±2.39	57.34±8.06**	67.67±4.39#&	69.06±3.87#&	83.05±3.67##
FS(%)	42.86±5.48	30.25±3.46**	37.23±4.36	38.31±4.91	47.73±8.39#
CO(%)	62.81±6.17	45.29±15.33	54.18±11.47	53.27±13.23	58.50±14.31
E/A ratio	1.11±0.09	1.85±0.40**	1.10±0.12##	1.14±0.11##	1.25±0.17##
IVRT(ms)	29.19±5.71	32.89±7.53	33.12±1.00	32.41±3.35	31.15±2.55

Note 3: One-way analysis of variance was performed; **, P < 0.01 compared to the CON group. #, P < 0.05; ##, P < 0.01 compared to the DCM group. &, P < 0.05 compared to the DLE group.

Comparison of the morphology of the rat myocardial tissue across the experimental groups

The myocardial tissues from each experimental group were stained with H&E and the tissue morphology was observed using a light microscope. In the control group, the myocardial cells were arranged neatly and densely, the myocardial fibers were tightly connected, the staining was uniform, and there was no muscle fiber dissolution. In the DCM group, the myocardium of rats showed damage and fractured myocardial fibers, the myocardial fibers were disordered, the muscle fiber gap was significantly widened, and there was blood cell accumulation in the interstitial space. In the DH, DL, and DLE groups, the myocardial fibers were neatly arranged without any breaks, the myocardial congestion was reduced, and the cell morphology was generally intact. The myocardial fibers in the DLE group were thicker and the arrangement was tighter than that in the DH and DL groups.(in Fig. 3)

Compared to the CON group, the total collagen in the myocardial tissue increased, the collagen arrangement was disordered, and a large number of collagen type I and III collagen fibers were dispersed around the cardiomyocytes in the DCM group. In the DLE group, the total collagen decreased significantly ($P < 0.05$). The total collagen content in the DL group was lower than that in the DH group ($P < 0.05$). There were no significant differences across the DLE, DL, and DH groups ($P > 0.05$).(in Fig. 4)

Comparison of the levels of myocardial AGEs across the experimental groups

After 8 weeks, the level of AGEs in the myocardium of the rats in the DCM group was significantly higher than that in the CON group ($P < 0.01$). Compared to the DCM group, the DH, DL, and DLE groups demonstrated significantly lower levels of AGEs ($P < 0.05$) and those in the DL group were slightly higher than those in the DH and DLE groups ,but there were no significant differences($P > 0.05$).(in Fig. 5)

Comparison of the expression of genes related to central ventricular remodeling in the myocardial tissue

The expression of ventricular remodeling genes *BNP*, *GSK3β*, *α-MHC*, and *β-MHC* in myocardial tissues was detected using RT-PCR (in Fig. 6).

After 8 weeks, the relative expression of *BNP* mRNA in the myocardium of the DCM group increased significantly compared to that in the CON group ($P < 0.01$). The DH, DL, and DLE groups showed a significant decrease in the relative expression of *BNP* mRNA ($P < 0.01$) compared to the DCM group but there were no significant differences across the DH, DL, and DLE groups ($P > 0.05$).

After 8 weeks, compared to the CON group, the relative expression of *GSK3β* mRNA in the myocardium of the DCM group increased significantly ($P < 0.01$). The DH, DL, and DLE groups demonstrated a significant decrease in the relative expression of *GSK3β* mRNA ($P < 0.01$) compared to the DCM group but there were no significant differences across the DH, DL, and DLE groups ($P > 0.05$).

After 8 weeks, compared to the CON group, the relative expression of *α-MHC* mRNA was significantly lower in the DCM group ($P < 0.05$). The relative expression of *α-MHC* mRNA was significantly increased in the DH, DL, and DLE groups compared to the DCM group ($P < 0.01$). The relative expression of *α-MHC* mRNA in the DLE group was significantly higher than that in the CON group ($P < 0.05$) but there were no significant differences across the DH, DL, and DLE groups ($P > 0.05$).

After 8 weeks, the DCM group demonstrated a significant increase in the relative expression of *β-MHC* mRNA compared to the CON group ($P < 0.01$). The relative expression of *β-MHC* mRNA decreased in the DH, DL, and DLE groups compared to the DCM group ($P < 0.01$) but there were no significant differences across the three groups ($P > 0.05$).

Comparison of GLP-1/GLP-1R protein expression in the myocardium across the experimental groups

After 8 weeks, the relative expression of GLP-1 protein in the myocardial tissue in the DCM group was significantly lower than that in the CON group ($P < 0.05$). The expression of GLP-1 significantly increased in the DH, DL, and DLE groups compared to the DCM group ($P < 0.01$). The expression of GLP-1 in the DH group was higher than that in the DL and DLE groups but the differences were not significant ($P > 0.05$). (in Fig. 7)

After 8 weeks, the expression of GLP-1R in myocardial tissue in the DCM group was lower than that in the CON group but the difference was not statistically significant ($P > 0.05$). The expression of GLP-1R increased significantly in the DH, DL, and DLE groups compared to that in the DCM ($P < 0.01$) and the CON groups. The DLE group showed significantly higher GLP-1R expression than the DH and DL groups but the differences across the DH, DL, and DLE groups were not significant ($P > 0.05$).

Analysis And Discussion

Liraglutide combined with AIT reduced the blood sugar levels in the DCM rats

The results of the current study showed that the blood glucose levels in the DH and DL groups decreased in the first week of the intervention followed by an insignificant increase from the second week, and then there was a small range within which the blood sugar level fluctuated. The blood glucose levels in the DLE group decreased throughout the 8 weeks. Fluctuations in blood glucose levels may cause an increase in the C-reactive protein levels, activate oxidative stress signaling pathways, directly damage cardiomyocytes, and lead to cardiomyocyte apoptosis [10]. Liraglutide and AIT markedly reduce HbA_{1c} levels, decrease insulin resistance, and improve pancreatic β-cell function to a certain extent. Administration of liraglutide promotes insulin secretion and inhibits the secretion of glucagon [11], thereby, lowering blood sugar levels. Exercise increases peripheral insulin sensitivity and glucose transport across the skeletal muscles via GLUT4 and reduces blood sugar levels [12]. In 2017, in a Danish

study, diabetic patients were given liraglutide therapy individually and in combination with aerobic exercise therapy. The results showed that the combined intervention restored the HbA_{1c} levels in diabetic patients [13]. In terms of the stability of the blood sugar levels, joint intervention not only decreases blood sugar quickly but also avoids the fluctuations therein.

Liraglutide combined with AIT reduced the AGEs and myocardial fibrosis and improved the cardiac structure in DCM rats

In a high-glucose environment, AGEs play an important role in the pathogenesis of diabetes.The accumulation of AGEs, and exercise reduces them [14]. The study by Panteleeva and Rogozkin showed that exercise significantly reduced the content of AGEs in diabetic rats [15]. The study by Di et al. also showed that liraglutide lowered the extent of phenotypic changes in the coronary artery smooth muscles caused by AGEs by inhibiting the NF-κB signaling pathway [16]. Exercise and liraglutide treatment reduce the content of AGEs in the body but there are no reports on the effect of the combination of the two on AGEs. The current study showed that diabetes leads to an increase in the content of AGEs in the myocardium and liraglutide combined with AIT significantly eliminated AGEs in the myocardium and maintained the stability of the extracellular matrix.

Disorders of the extracellular matrix (ECM) can cause cardiac dysfunction. Collagen is the main component of myocardial ECM and the hyperplasia and remodeling of collagen fibers are closely related to ventricular remodeling. In the current study, Picrosirius red staining showed increased collagen accumulation in the DCM myocardium and collagen type I and type III hyperplasia. Zhao et al. showed that liraglutide protected DCM from myocardial fibrosis by activating the CD36-JNK-AP1 signaling pathway and down-regulating P4HA1 [17]. Chen et al. showed that liraglutide reduced the level of myocardial fibrosis in hypertensive mice by inhibiting the production of ROS [18]. Another study confirmed that exercise training reduces collagen accumulation in the myocardium of diabetic rats [19]. Treadmill exercises for 4 weeks markedly reduced the accumulation of collagen in the myocardium and lowered the expression of type Ⅰ and type Ⅲ collagen [20]. The results of our current study were consistent with these aforementioned results. After the 8-week intervention of liraglutide combined with AIT, type I and type III collagen in the myocardium reduced, indicating that the process of myocardial fibrosis can be effectively controlled to protect the heart.

Glycogen synthase kinase-3 (GSK-3) is a widely expressed and highly conserved serine/threonine kinase. In addition to regulating glycogen synthesis, GSK3β is involved in the pathogenesis of diabetes and the progression of myocardial fibrosis [21]. The expression of GSK3β in the myocardium of STZ-induced diabetic rats increased significantly after 28 days [22], which was consistent with the results of our study. Evidence suggests that GSK3β regulates the SMAD-3/TGF-β1[23]signaling pathway and activates the AMPK/GSK3β/NFR2 signaling pathway to achieve the anti-fibrotic effect and alleviate cardiomyocyte apoptos. It can decrease atrial natriuretic peptide and markedly improve the left ventricular function to

protect the morphology and function of the heart. [24, 25] The results of the current study show that liraglutide alone and in combination with AIT reduced the expression of myocardial GSK3β and inhibited the accumulation of myocardial collagen fibers, thereby alleviating myocardial fibrosis and protecting the diabetic heart.

Liraglutide combined with AIT increased myocardial contractility and improved cardiac function

The initial damage to the myocardium due to diabetes affects the diastolic function, and as the disease progresses, systolic dysfunction develops [26]. The occurrence of heart failure is due to the primary or secondary weakening of myocardial contractility, which is related to the structure of tropomyosin. The head of the tropomyosin is composed of a myosin heavy chain (MHC), which is divided into α and β subunits. The ATPase activity of the α subunit is higher than that of the β subunit [27]. Rundell et al. showed that the expression of β-MHC increased and that of α-MHC decreased in STZ-induced animals, decreasing the tension of myocardial fiber and myocardial contractility [28]. In the current study, the changes in the heavy chain subtypes of tropomyosin in the myocardium of diabetic rats affected the normal excitation-contraction coupling, which corresponded to a dysfunction in the myocardial contraction as shown by a decrease in LVEF and FS. In diabetic rats, insulin or carnitine linear transferase inhibitor improved the expression of myocardial α-MHC [29]. Exercise intervention reduced myocardial fibrosis and apoptosis by reducing the expression of β-MHC [30]. Liraglutide, alone and in combination with AIT, significantly decreased the relative expression of *β-MHC* mRNA and increased that of *α-MHC* mRNA. Exercise training may have improved the excitability of sympathetic nerves and acetylcholine promoting the expression of *α-MHC* mRNA [31]. On the one hand, exercise stress increases the relative expression of *α-MHC* mRNA and myocardial contractility in the heart. On the other hand, it increases the traction on the heart, resulting in compression of the ventricular wall, which increases BNP.

BNP is produced by the ventricle. It is produced when the ventricular wall is compressed during ventricular pressure or volume overload. As a grade I risk factor for heart failure, the progression of diabetes is positively correlated with BNP [32]. When the duration of diabetes in mice reached 2 months, the BNP level increased [33]. The results of our study are consistent with these. In our study, the serum BNP levels of diabetic rats were significantly higher than that of the control rats after 10 weeks of establishing the diabetic model. GLP-1 significantly reduced the level of BNP in patients with type 2 diabetes [34]. The results of our study are consistent with these results. A meta-analysis showed that both aerobic and resistance exercises reduced the level of BNP in patients with heart failure [35]. Our study showed that 8 weeks of liraglutide intervention significantly reduced the level of BNP in the myocardium of diabetic rats. Compared to the DH and DL groups, the intramyocardial BNP levels in the DLE group were slightly higher. Although there were no significant differences across the DH, DL, and DLE groups, the three groups showed a significant decline in the myocardial BNP levels of DCM rats.

Therefore, liraglutide intervention in combination with AIT, on the one hand, increases the relative expression of *α-MHC* mRNA and myocardial contractility, and on the other, reduces the strain on the

myocardium due to the excitement of the sympathetic nerve due to exercise. It also decreases the blood BNP levels, showing a neutralizing effect.

Liraglutide combined with AIT activated the GLP-1/GLP-1R signaling pathway and protected the DCM heart

Liraglutide is produced by the non-covalent binding of GLP-1 and albumin [36]. The half-life of liraglutide is relatively long, up to 13 hours [37]. In the diabetic patients who were administered liraglutide, the left ventricular mass index was significantly lesser than that in the control group, and the left ventricular end-systolic volume and left ventricular end-diastolic volume were lower, suggesting that liraglutide prevents left ventricular remodeling in diabetic patients [38]. Noyan-Ashraf and others tested the proteins that regulate myocardial survival [39]. The study found that 200 μg liraglutide injection twice a day for 1 week increased the activities of AKT, GSK3β, and PPARα, thereby improving the survival of diabetic mice after myocardial infarction, and this effect was independent of liraglutide's weight-lowering and blood-sugar-lowering effects. This result was consistent with the results of our study. The expression levels of GLP-1 and GLP-1R in the myocardial tissue of diabetic rats were significantly lower than those in the control group. Both high and low doses of liraglutide increased the expression of GLP-1 and GLP-1R and decreased the level of GSK3β in the myocardium. The level of GLP-1 in the high-dose group was higher than that in the low-dose group but the GLP-1R level was not significantly different from that in the low-dose group.

Exercise increased the expression of GLP-1 and GLP-1R in the myocardial tissue. Studies have shown that treadmill exercise for 8 weeks contributed to a significant rise in the expression of GLP-1 and GLP-1R in the myocardial tissue of diabetic rats. The results of our study are consistent with these aforementioned results. Exercise training increased the expression of GLP-1R. Systematic exercise training may increase the sensitivity of hormone receptors [40]. Liraglutide acts as a GLP-1R agonist and exerts biological effects through GLP-1R. Using cell culture experiments, we can check whether the expression of GLP-1R increases as the concentration of GLP-1 increases. However, in the current study, the expression levels of GLP-1R in the high-dose and low-dose groups of liraglutide were inconsistent with those of GLP-1, which may be due to receptor desensitization. GPCRs bind to the receptor but the short-term or long-term attenuation of the cell signal response is due to receptor desensitization [41]. This partly explains why there were no significant differences related to improvement in the heart function between high- and low-dose liraglutide groups. This also explains the clinical resistance that arises during the treatment of diabetes, and the need to continuously increase the dose of medicine to maintain a stable blood sugar level. When liraglutide is combined with AIT, the expression of GLP-1R increased significantly, indicating that exercise improves receptor sensitivity. Therefore, the combined intervention shows complementary advantages in decreasing myocardial lipids, fibrosis, and myocardial hypertrophy and improving cardiac function.

Conclusion

Liraglutide combined with AIT intervention significantly reduced myocardial fibrosis, increased the expression of GLP-1/GLP-1R in the myocardium, myocardial contractility, and cardiac function, and improved the morphology and functioning of the heart in 8 weeks.

Experimental Subjects And Methods

Model and grouping

The study was conducted in accordance with the Hebei Province Experimental Animal Management Regulations and Use of Laboratory Animals and the protocol was approved by the Animal Ethics Committee of the Hebei General Hospital(NO.2020101),and every effort was made to minimize both the number of animals used and their suffering. All the experiments were also performed and reported in accordance with the ARRIVE guidelines 2.0.Wistar rats (8 weeks old, bodyweight 250-280 g) were divided into 5 groups - normal control group (CON, n = 10), diabetic cardiomyopathy group (DCM, n = 10), high-dose liraglutide group (DH, n = 10), low-dose liraglutide group (DL, n = 10), and aerobic interval training combined with liraglutide group (DLE, n = 10). After feeding them with high-fat for 4 weeks, a small dose of STZ (35 mg/kg) was injected intraperitoneally, and two consecutive fasting blood glucose (FBG) measurements ≥ 11.1 mmol/L indicated that the diabetes model was established. After establishing the diabetes model, the drug and exercise intervention was conducted for 8 weeks. During the intervention, the CON group was fed ordinary feed and the rest of the groups were fed a high-fat diet *ad libitum*.

Intervention plan

Drug Intervention Program

The rats in the DH group were administered 0.4 ml/kg/day liraglutide and those in the DL and DLE groups were administered 0.2 ml/kg/day liraglutide. An equal volume of saline was administered to the rats in the CON group for 8 weeks.(in Fig. 1)

Exercise intervention program

The exercise intervention program adopted AIT using a treadmill. The exercise time was 60 min and it was set during the dark cycle, in consideration of the animals' biological rhythm, from 20 o'clock to 21 o'clock. The rats were provided 1 week of adaptive training, and AIT training was initiated in the second week. The exercise intensity was 25 m/min and the exercise time was 7 min. The intermittent period was active rest at 15 m/min for 3 min and was repeated 4 times.

Echocardiography

Routine echocardiographic examination of rats was performed after exercise intervention [9], and left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic diameter (LVEDD), left ventricular ejection fraction (LVEF), fractional shortening (FS), cardiac output (CO), E/A ratio, and isovolumetric relaxation time (IVRT) were determined.

Blood index test

Tail vein blood was collected before the intervention (wk0), and in the first (wk1), second (wk2), fourth (wk4), sixth (wk6), and eighth week (wk8) of the intervention to test the FBG. Blood was drawn from the heart apex. It was centrifuged at a low temperature at 3500 rpm for 15 min to separate the serum and stored at -20°C. The levels of FBG, FINs, and HbA_{1c} were measured. Insulin resistance (HOMA-IR) and beta-cell function (HOMA-β) were calculated using the following equations -

HOMA-IR = FBG × FINS/22.5 and

HOMA-β = (FINS × 20)/(FBG - 35).

Histological examination

After the blood was drawn, starting from the aorta, the circulatory system was flushed with pre-cooled normal saline containing diethyl pyrocarbonate (DEPC). The heart was removed, and 5 mm^3 of the myocardial tissue from the upper and lower midpoints of the symmetrical plane perpendicular to the long axis of the left ventricle of the heart was resected. Then the tissue was fixed with 4% paraformaldehyde, embedded in paraffin, and stained with H&E and Sirius red.

Tissue homogenate testing

About 50-mg tissue at the apex of the rat heart was resected and rinsed with pre-cooled saline. Then, 500 µl saline was added, and 10% of the tissue was homogenized using a homogenizer (IKA Tlobasic, Germany) and an ultrasonic cell crusher (Scientz-IID, Xinzhi, Ningbo, China). The homogenate was centrifuged at 3500 rpm for 15 min at 4°C. Then, the supernatant was aspirated, and the amount of advanced glycation end products (AGEs) was determined.

Real-time quantitative PCR detection of ventricular remodeling genes

Myocardial tissue (100 mg) was resected and 1 ml of trizol reagent was added to extract the RNA. First Strand cDNA Synthesis Kit (Thermo) was used to obtain cDNA according to the manufacturer's instructions. Then, real-time PCR was performed using SYBR Green (Roche) using Mastercycler ep realplex PCR instrument (Eppendorf), where the reaction system was 25 µl. Each sample was subjected to real-time PCR in triplicates. The primer sequences used for PCR amplification are shown (in Table 1).

Table 1
Sequence of Primers Detected using Real-Time PCR

Gene	Primer Sequence	Amplified Fragment Length
α-MHC	Forward: 5’-AAGAAGAACTTGGTGCGGCT-3’	234bp
	Reverse: 5’-ATCGTGCATTTTCTGCTTGGC-3’	
β-MHC	Forward: 5’-GCACCGTGGACTACAATATCCT-3’	117bp
	Reverse: 5’-TGGCAAACAGATTACTTAGGAGC-3’	
GSK3β	Forward: 5’-ATTCCCTCAAATTAAGGCACATCC-3’	133bp
	Reverse: 5’-ATACTCCAGCAGAGGGGCTACACAG-3’	
BNP	Forward: 5’ -TCCTGCTTTTCCTTAATCTGTCG-3’	258bp
	Revrese: 5’ -AGCTTGAACTATGTGCCATCTTG-3’	
β-tublin	Forward: 5’-CGAGAAGAATACCCCGACCG-3’	115bp
	Reverse: 5’-CTACCAACTGGTGGACGGAC-3’	

Western blotting was performed to measure the expression of myocardial GLP-1/GLP-1R

Myocardial tissue (30 mg) was treated with 300 μl of RIPA lysis buffer to extract the total protein in the myocardial cells. Protein concentration was determined using the BCA method. About 20 μg protein was added to each well and SDS-PAGE electrophoresis was performed. The bands were transferred 300 mA to polyvinylidene fluoride (PVDF) membranes. Primary antibodies against GLP-1 (1:500), GLP-1R (1:100), and β-tubulin (1:1000) were added and the membranes were incubated at 4°C overnight[9].The secondary antibody, diluted 1:3000, was added and the membranes were incubated at room temperature for 2 h. Chemiluminescence (ECL) imaging was performed. ImageJ was used to analyze the images, and β-tubulin was used as an internal reference to compare the optical density values across the groups of bands.

Statistical analysis

SPSS 20.0 was used for statistical analysis. The Schapiro-Wilk normal distribution test was performed. Data are expressed as mean ± standard deviation (M ± SD). ANOVA was used for comparison between groups. Significance thresholds used were $P < 0.05$ and $P < 0.01$.

Ethics Statement

The study was conducted in accordance with the Hebei Province Experimental Animal Management Regulations and Use of Laboratory Animals and the protocol was approved by the Animal Ethics Committee of the Hebei General Hospital(NO.2020101),and every effort was made to minimize both the

number of animals used and their suffering. All the experiments were also performed and reported in accordance with the ARRIVE guidelines 2.0.

Declarations

Acknowledgments

The authors thank the field workers for their contribution and the participants for their cooperation.This research was supported by& Liraglutide promotes the& White Fat Browning in obese through the down-regulates& micro RNA 27b. Hebei Provincial Natural Science Foundation (H2019307112).

Author& Contributions Statement

Conceptualization, H.C, J.Q.L; Investigation, H.C,J.Q.L; Writing – Original Draft, H.C,L.L.Z,Z.L.L; Writing – Review & Editing,H.C,L.L.Z, S.C.C; Supervision, S.C.C.

Disclosures

The authors declare that they have no conflicts of interest.

References

1. Zhang L, Tian J, Diao S, Zhang G, Xiao M, Chang D. Glp-1 receptor agonist liraglutide protects cardiomyocytes from il-1beta-induced metabolic disturbance and mitochondrial dysfunction. Chem Biol Interact 2020;332:109252.
2. Jakubik D, Fitas A, Eyileten C, Jarosz-Popek J, Nowak A, Czajka P, et al. Micrornas and long non-coding rnas in the pathophysiological processes of diabetic cardiomyopathy: Emerging biomarkers and potential therapeutics. Cardiovasc Diabetol 2021;20(1):55.
3. Almutairi M, Al Batran R, Ussher JR. Glucagon-like peptide-1 receptor action in the vasculature. Peptides 2019;111:26–32.
4. Garber AJ, Abrahamson MJ, Barzilay JI, Blonde L, Bloomgarden ZT, Bush MA, et al. Consensus statement by the american association of clinical endocrinologists and american college of endocrinology on the comprehensive type 2 diabetes management algorithm - 2018 executive summary. Endocr Pract 2018;24(1):91–120.
5. Vitz M, Moss B, Ward H, Soma P, Isichei-Kizito J, Hall R, et al. New zealand may finally get funded access to diabetes drugs which reduce cardiovascular events and progression of kidney disease: An audit of proposed pharmac criteria compared with international guidelines. N Z Med J 2020;133(1523):76–86.
6. Chinese Medical Association Diabetes Branch, The guidelines for the prevention and control of type 2 diabetes in China (2020 Edition). Chinese Journal of Diabetes,13(04): 315–409

7. Konopka AR, Laurin JL, Schoenberg HM, Reid JJ, Castor WM, Wolff CA, et al. Metformin inhibits mitochondrial adaptations to aerobic exercise training in older adults. Aging Cell 2019;18(1):e12880.
8. Piao SJ, Kim SH, Suh YJ, Hong SB, Ahn SH, Seo DH, et al. Beneficial effects of aerobic exercise training combined with rosiglitazone on glucose metabolism in otsuka long evans tokushima fatty rats. Diabetes Metab J 2017;41(6):474–85.
9. Wang LW, Kesteven SH, Huttner IG, Feneley MP, Fatkin D. High-frequency echocardiography-transformative clinical and research applications in humans, mice, and zebrafish. Circ J 2018;82(3):620–28.
10. Kar S, Kambis TN, Mishra PK. Hydrogen sulfide-mediated regulation of cell death signaling ameliorates adverse cardiac remodeling and diabetic cardiomyopathy. Am J Physiol Heart Circ Physiol 2019;316(6):H1237-52.
11. Dang-Tan T, Kamble PS, Meah Y, Gamble C, Ganguly R, Horter L. Real-world effectiveness of liraglutide vs. Sitagliptin among older patients with type 2 diabetes enrolled in a medicare advantage prescription drug plan: A retrospective observational study. Diabetes Ther 2020;11(1):213–28.
12. Wang Y, Wen L, Zhou S, Zhang Y, Wang XH, He YY, et al. Effects of four weeks intermittent hypoxia intervention on glucose homeostasis, insulin sensitivity, glut4 translocation, insulin receptor phosphorylation, and akt activity in skeletal muscle of obese mice with type 2 diabetes. Plos One 2018;13(9):e0203551.
13. Mensberg P, Nyby S, Jorgensen PG, Storgaard H, Jensen MT, Sivertsen J, et al. Near-normalization of glycaemic control with glucagon-like peptide-1 receptor agonist treatment combined with exercise in patients with type 2 diabetes. Diabetes Obes Metab 2017;19(2):172–80.
14. Oushah H. The influence of exercise, smoking cessation and ageing on the level of advance glycation endproducts: Manchester Metropolitan University; 2016.
15. Panteleeva IG, Rogozkin VA. Effect of physical load on serum protein glycation in rats with induced diabetes. Ross Fiziol Zh Im I M Sechenova 2001;87(9):1202-7. (in Russian)
16. Di B, Li HW, Li W, Hua B. Liraglutide inhibited ages induced coronary smooth muscle cell phenotypic transition through inhibiting the nf-kappab signal pathway. Peptides 2019;112:125–32.
17. Zhao T, Chen H, Cheng C, Zhang J, Yan Z, Kuang J, et al. Liraglutide protects high-glucose-stimulated fibroblasts by activating the cd36-jnk-ap1 pathway to downregulate p4ha1. Biomed Pharmacother 2019;118:109224.
18. Chen P, Yang F, Wang W, Li X, Liu D, Zhang Y, et al. Liraglutide attenuates myocardial fibrosis via inhibition of at1r-mediated ros production in hypertensive mice. J Cardiovasc Pharmacol Ther 2021;26(2):179–88.
19. Castellar A, Remedio RN, Barbosa RA, Gomes RJ, Caetano FH. Collagen and reticular fibers in left ventricular muscle in diabetic rats: Physical exercise prevents its changes? Tissue Cell 2011;43(1):24–8.
20. Silva FS, Bortolin RH, Araujo DN, Marques DES, Lima J, Rezende AA, et al. Exercise training ameliorates matrix metalloproteinases 2 and 9 messenger rna expression and mitigates adverse left

ventricular remodeling in streptozotocin-induced diabetic rats. Cardiovasc Pathol 2017;29:37–44.

21. Potz BA, Scrimgeour LA, Sabe SA, Clements RT, Sodha NR, Sellke FW. Glycogen synthase kinase 3beta inhibition reduces mitochondrial oxidative stress in chronic myocardial ischemia. J Thorac Cardiovasc Surg 2018;155(6):2492–503.
22. Gurusamy N, Watanabe K, Ma M, Prakash P, Hirabayashi K, Zhang S, et al. Glycogen synthase kinase 3beta together with 14-3-3 protein regulates diabetic cardiomyopathy: Effect of losartan and tempol. FEBS Lett 2006;580(8):1932–40.
23. Guo Y, Gupte M, Umbarkar P, Singh AP, Sui JY, Force T, et al. Entanglement of gsk-3beta, beta-catenin and tgf-beta1 signaling network to regulate myocardial fibrosis. J Mol Cell Cardiol 2017;110:109–20.
24. Fujita A, Takahashi-Yanaga F, Morimoto S, Yoshihara T, Arioka M, Igawa K, et al. 2,5-dimethylcelecoxib prevents pressure-induced left ventricular remodeling through gsk-3 activation. Hypertens Res 2017;40(2):130–39.
25. Duan J, Guan Y, Mu F, Guo C, Zhang E, Yin Y, et al. Protective effect of butin against ischemia/reperfusion-induced myocardial injury in diabetic mice: Involvement of the ampk/gsk-3beta/nrf2 signaling pathway. Sci Rep 2017;7:41491.
26. Nikolajevic Starcevic J, Janic M, Sabovic M. Molecular mechanisms responsible for diastolic dysfunction in diabetes mellitus patients. Int J Mol Sci 2019;20(5):1197.
27. Gupta MP. Factors controlling cardiac myosin-isoform shift during hypertrophy and heart failure. J Mol Cell Cardiol 2007;43(4):388–403.
28. Rundell VLM, Geenen DL, Buttrick PM, de Tombe PP. Depressed cardiac tension cost in experimental diabetes is due to altered myosin heavy chain isoform expression. Am J Physiol-Heart C 2004;287(1):H408-13.
29. Dillmann WH. Methyl palmoxirate increases ca2+-myosin atpase activity and changes myosin isoenzyme distribution in the diabetic rat heart. Am J Physiol 1985;248(5 Pt 1):E602-6.
30. Konhilas JP, Watson PA, Maass AH, Boucek DM, Horn T, Stauffer BL, et al. Exercise can prevent and reverse the severity of hypertrophic cardiomyopathy. Circ Res 2006;98(4):540–48.
31. Pauletto P, Dalla Libera L, Vescovo G, Scannapieco G, Angelini A, Pessina AC, et al. Propranolol-induced changes in ventricular isomyosin composition in the rat. Am Heart J 1985;109(6):1269–73.
32. Birukov A, Eichelmann F, Kuxhaus O, Polemiti E, Fritsche A, Wirth J, et al. Opposing associations of nt-probnp with risks of diabetes and diabetes-related complications. Diabetes Care 2020;43(12):2930–37.
33. Sen S, Chen S, Feng B, Iglarz M, Chakrabarti S. Renal, retinal and cardiac changes in type 2 diabetes are attenuated by macitentan, a dual endothelin receptor antagonist. Life Sci 2012;91(13-14):658–68.
34. Halbirk M, Norrelund H, Moller N, Holst JJ, Schmitz O, Nielsen R, et al. Cardiovascular and metabolic effects of 48-h glucagon-like peptide-1 infusion in compensated chronic patients with heart failure. Am J Physiol Heart Circ Physiol 2010;298(3):H1096-102.

35. Smart NA, Meyer T, Butterfield JA, Faddy SC, Passino C, Malfatto G, et al. Individual patient meta-analysis of exercise training effects on systemic brain natriuretic peptide expression in heart failure. Eur J Prev Cardiol 2012;19(3):428–35.
36. Han J, Huang Y, Chen X, Zhou F, Fei Y, Fu J. Rational design of dimeric lipidated xenopus glucagon-like peptide 1 analogues as long-acting antihyperglycaemic agents. Eur J Med Chem 2018;157:177–87.
37. Han J, Chen X, Zhao L, Fu J, Sun L, Zhang Y, et al. Lithocholic acid-based peptide delivery system for an enhanced pharmacological and pharmacokinetic profile of xenopus glp-1 analogs. Mol Pharm 2018;15(7):2840–56.
38. Nozue T, Yamada M, Tsunoda T, Katoh H, Ito S, Iwaki T, et al. Effects of liraglutide, a glucagon-like peptide-1 analog, on left ventricular remodeling assessed by cardiac magnetic resonance imaging in patients with acute myocardial infarction undergoing primary percutaneous coronary intervention. Heart Vessels 2016;31(8):1239–46.
39. Noyan-Ashraf MH, Momen MA, Ban K, Sadi AM, Zhou YQ, Riazi AM, et al. Glp-1r agonist liraglutide activates cytoprotective pathways and improves outcomes after experimental myocardial infarction in mice. Diabetes 2009;58(4):975–83.
40. Wojtaszewski JF, Hansen BF, Gade, Kiens B, Markuns JF, Goodyear LJ, et al. Insulin signaling and insulin sensitivity after exercise in human skeletal muscle. Diabetes 2000;49(3):325–31.
41. Gurevich VV, Gurevich EV. The structural basis of arrestin-mediated regulation of g-protein-coupled receptors. Pharmacol Ther 2006;110(3):465–502.

Figures

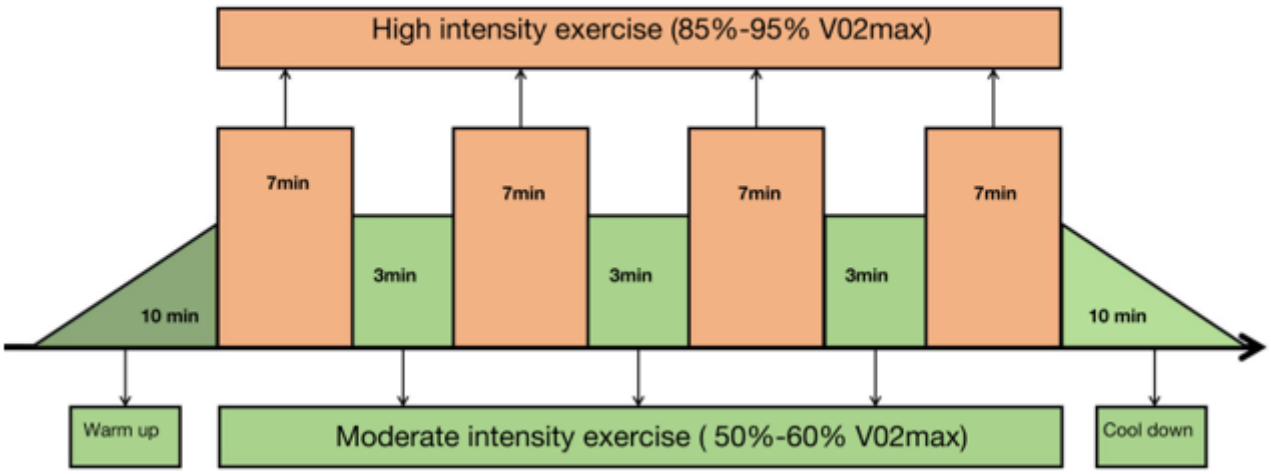

Figure 1

AIT program

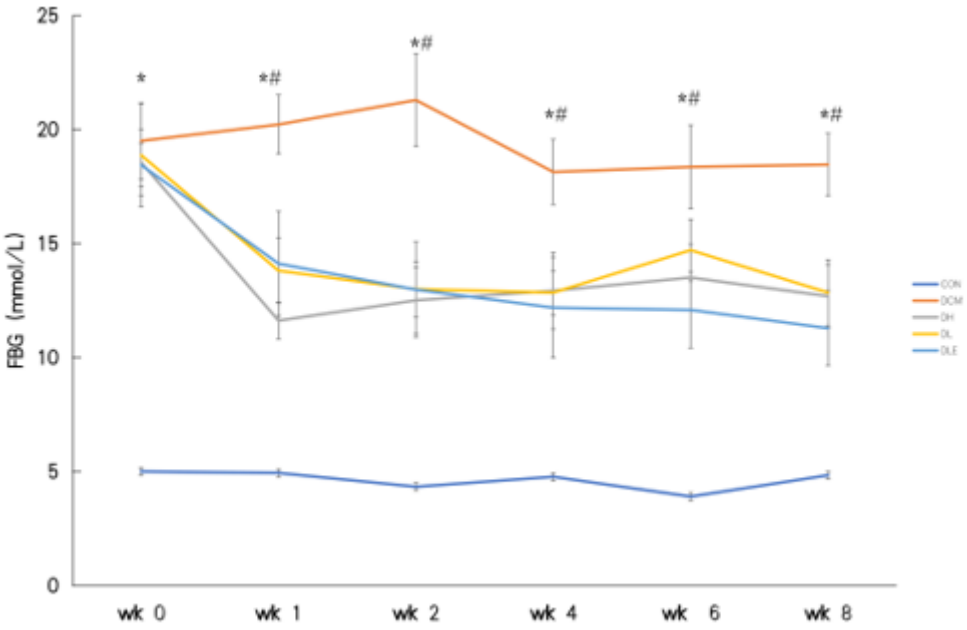

Figure 2

The changes in FBG levels in the experimental groups during the intervention

Note 1: One-way analysis of variance was performed; *, P < 0.05 compared to the CON group; #, P < 0.05 compared to the DCM group.

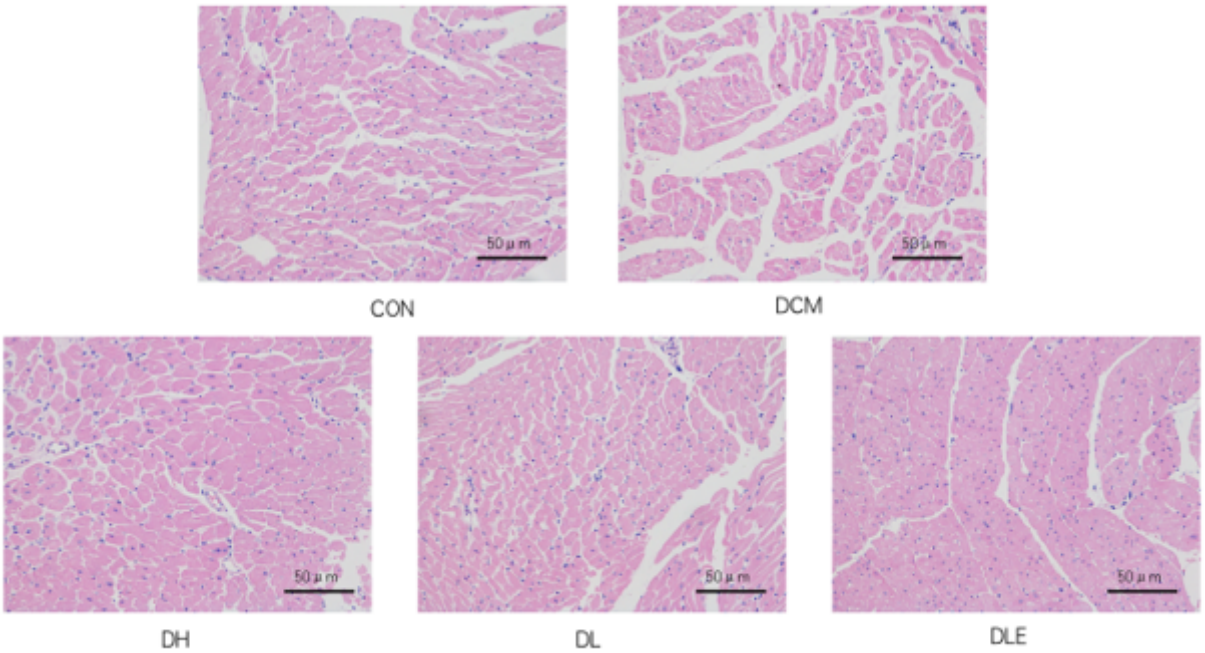

Figure 3

Comparison of myocardial tissue morphology across the experimental groups

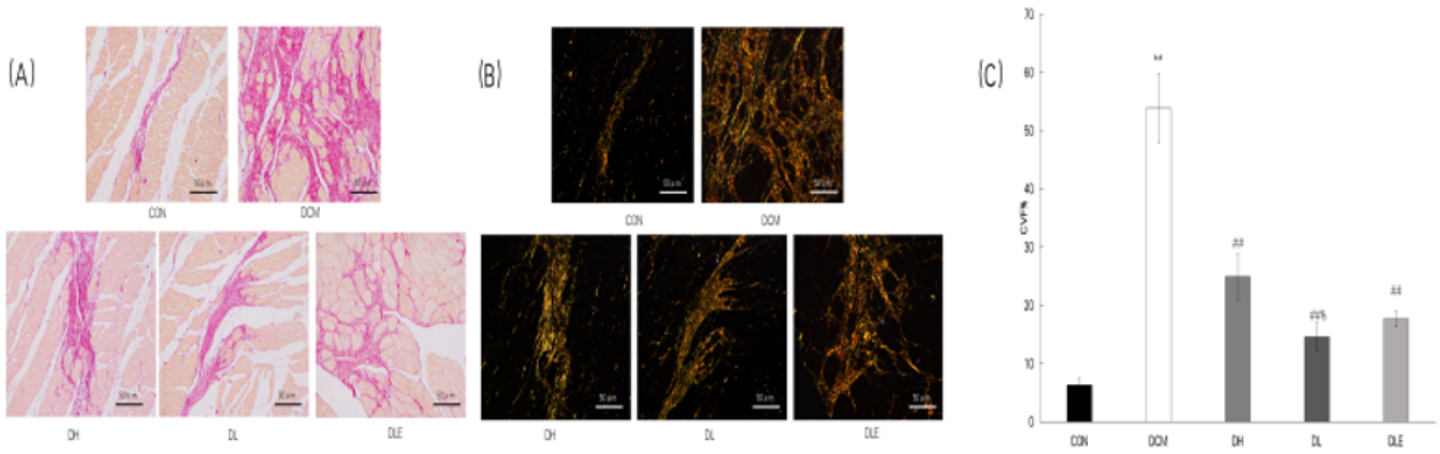

Figure 4

Detection of myocardial fibrosis in rats. Note 4: (A) The myocardial tissue stained using Picrosirius red and observed using white light (200×) to evaluate collagen distribution. (B) The myocardial tissue stained using Picrosirius red and observed using polarized light (200×) to evaluate the distribution of types I and III collagen. (C)CVF,Cyclophosphamide+Vinorelbine+5-Fluorouracil.One-way analysis of variance was performed; **, P < 0.01 compared to the CON group; ##, P < 0.01 compared to the DCM group; %, P < 0.05 compared to the DH group.

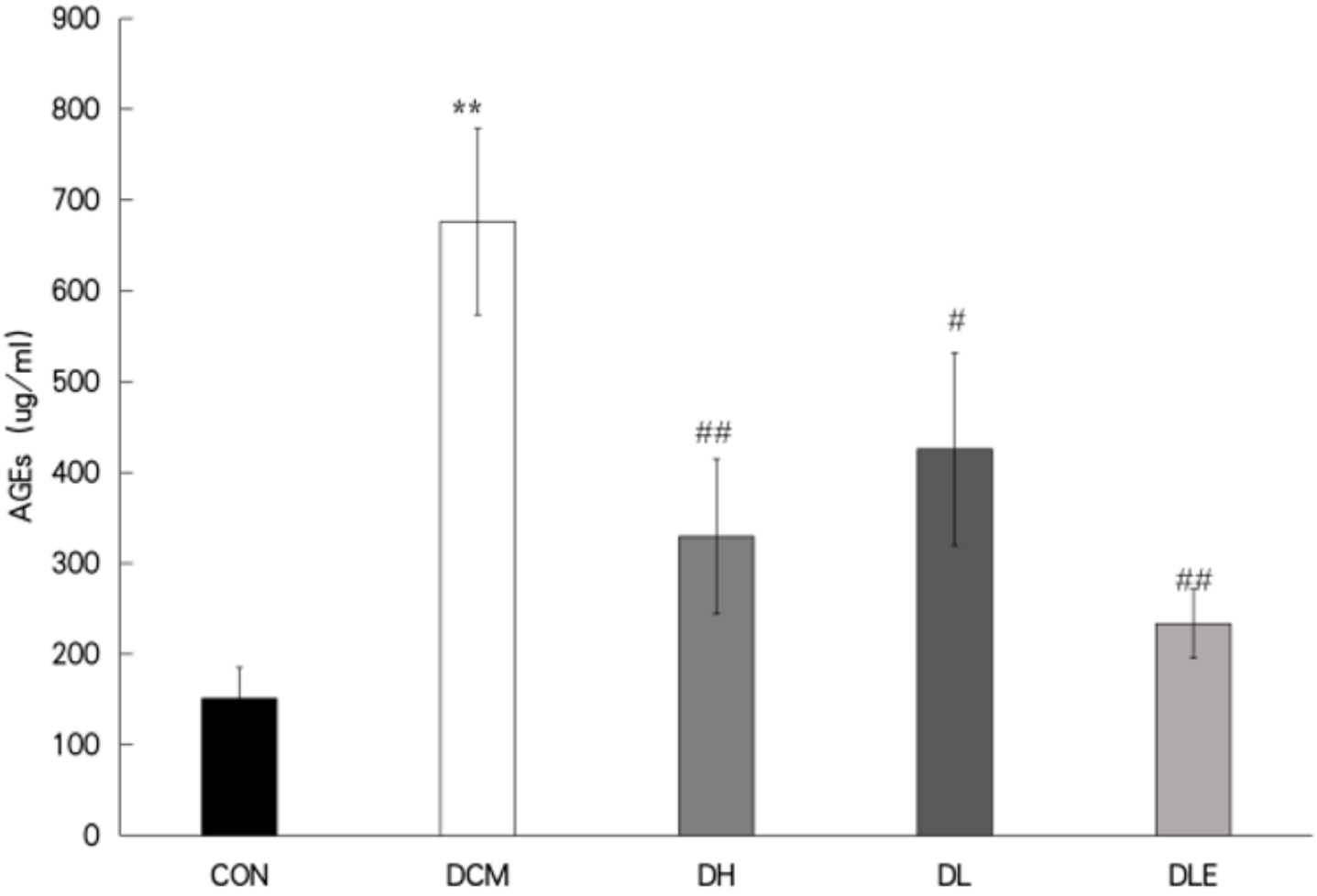

Figure 5

Comparison of the levels of cardiac AGEs across the experimental groups. Note 5: One-way analysis of variance was performed. **, P < 0.01 compared to the CON group. #, P < 0.05; ##, P < 0.01 compared to the DCM group.

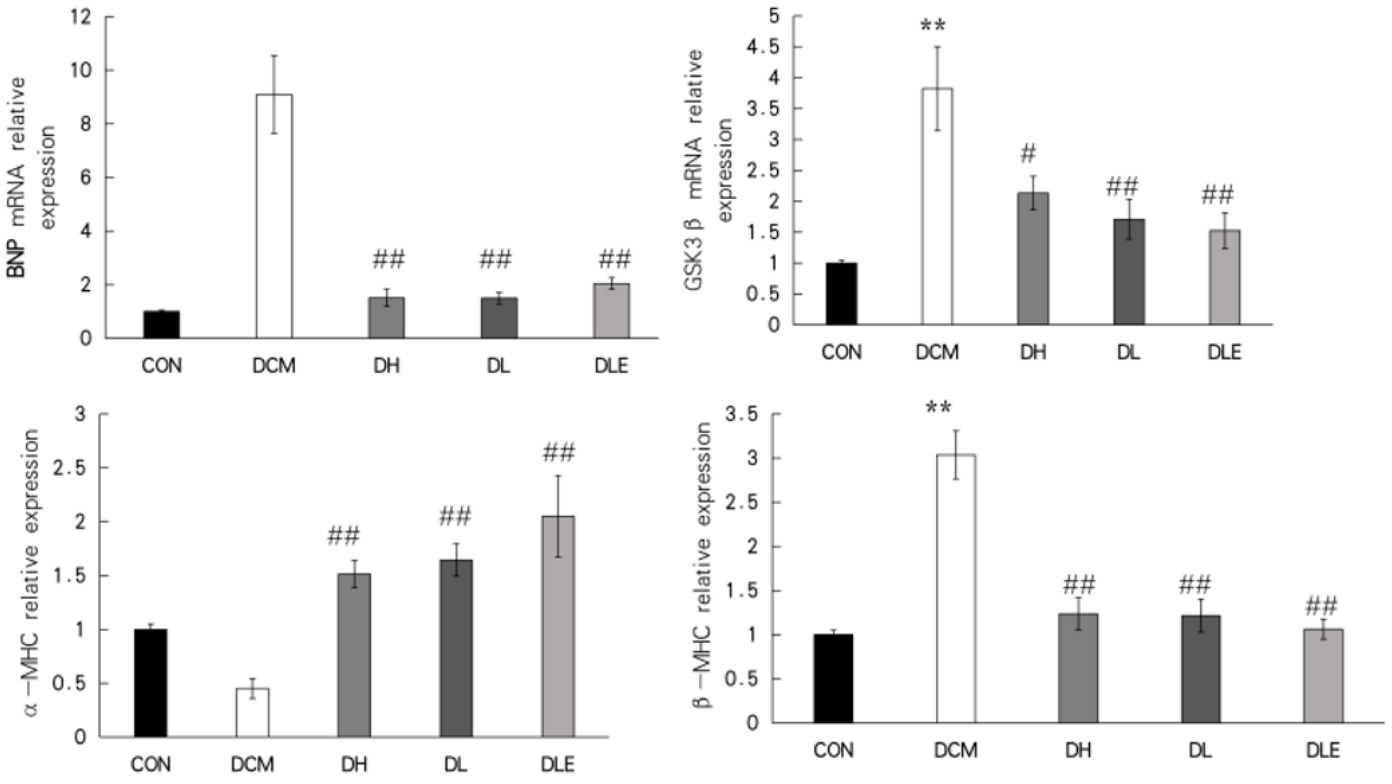

Figure 6

The relative mRNA expression of *BNP*, *GSK3β*, *α-MHC*, and *β-MHC* across the experimental groups. Note 6: One-way analysis of variance was performed; **, P < 0.01 compared to the CON group; ##, P < 0.01；#P ＜0.05 compared to the DCM group.

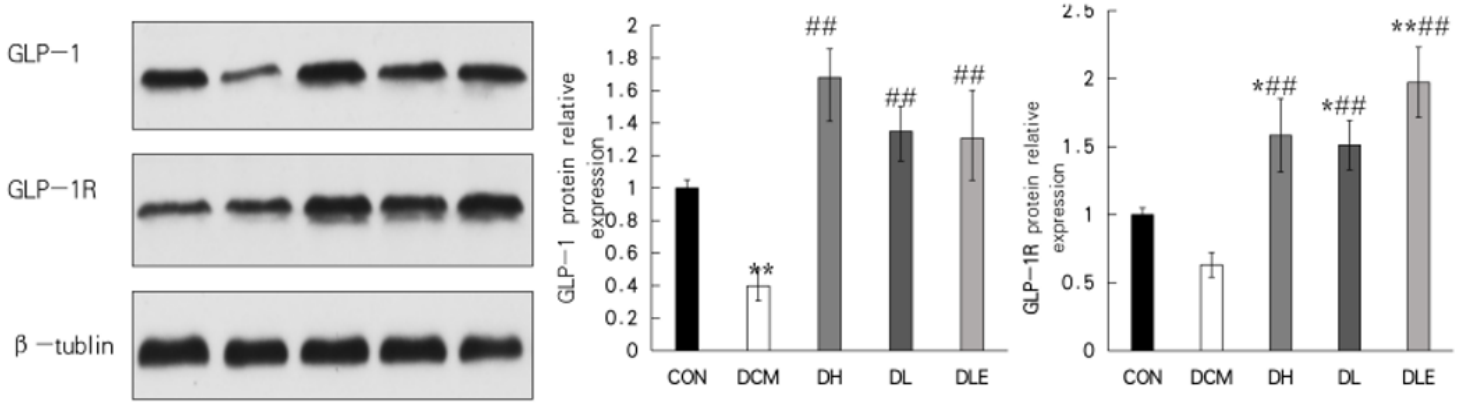

Figure 7

The relative expression of GLP-1 and GLP-1R across the experimental groups. Note 7: One-way analysis of variance was performed. *, P < 0.05; **, P < 0.01 compared to the CON group. ##, P < 0.01 compared to the DCM group.