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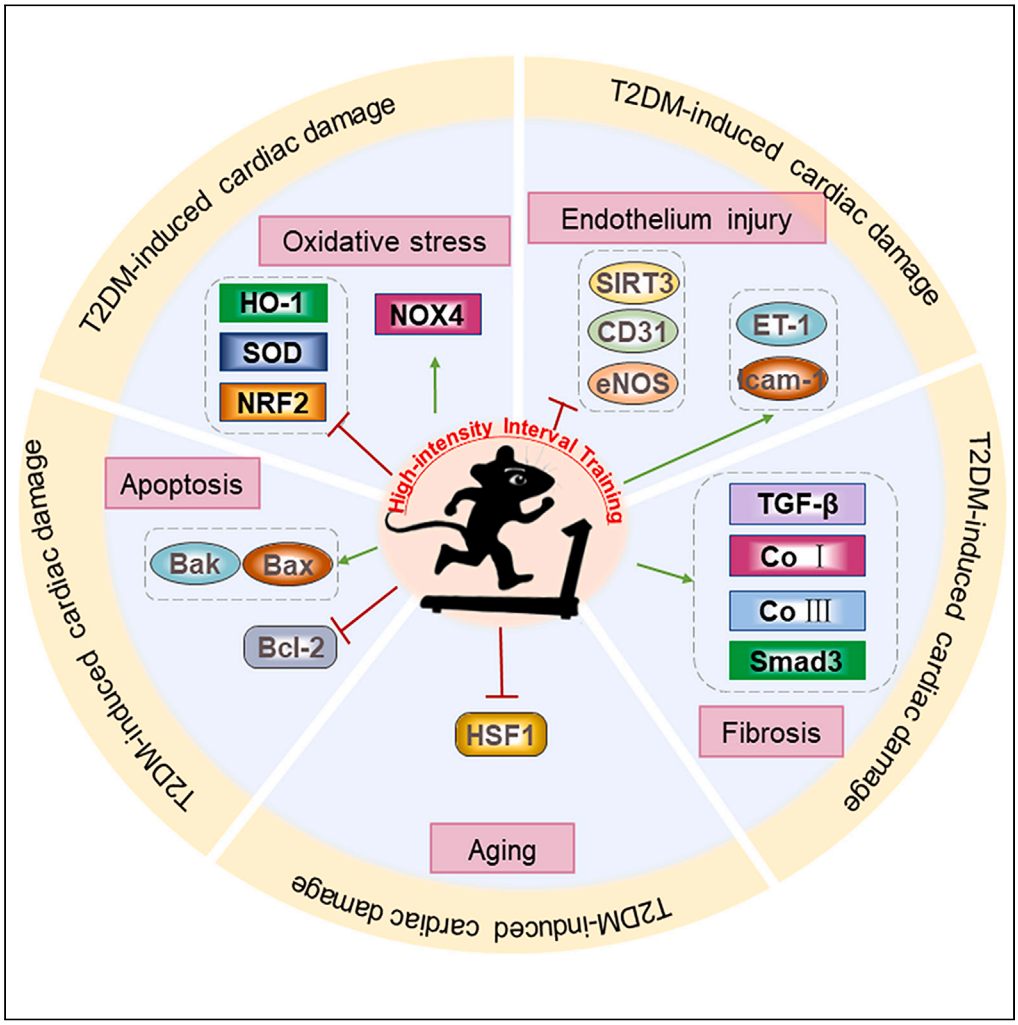

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Highlights

Exercise is the primary prevention of T2DM

HIIT decreases myocardial remodeling, fibrosis, oxidative stress and apoptosis

HIIT is superior to MICT on reducing myocardial injury in T2DM mice

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Different exercise training intensities prevent type 2 diabetes mellitus-induced myocardial injury in male mice

Zuowei Pei,[1,2,5,*] Rui Zhou,[3] Wei Yao,[3] Shuang Dong,[1] Yingshu Liu,[4] and Zhengnan Gao[4,*]

SUMMARY

Type 2 diabetes mellitus (T2DM) usually develop myocardial injury and that exercise may have a positive effect on cardiac function. However, the effect of exercise intensity on cardiac function has not yet been fully examined. This study aimed to explore different exercise intensities on T2DM-induced myocardial injury. 18-week-old male mice were randomly divided into four groups: a control group, the T2DM, T2DM + medium-intensity continuous training (T2DM + MICT), and T2DM + high-intensity interval training (T2DM + HIIT) groups. In the experimental group, mice were given high-fat foods and streptozotocin for six weeks and then divided into two exercise training groups, in which mice were subjected to exercise five days per week for 24 consecutive weeks. Finally, metabolic characteristics, cardiac function, myocardial remodeling, myocardial fibrosis, oxidative stress, and apoptosis were analyzed. HIIT treatment improved cardiac function and improved myocardial injury. In conclusion, HIIT may be an effective means to guard against T2DM-induced myocardial injury.

INTRODUCTION

Diabetes mellitus (DM) is considered the most significant challenge for healthcare systems, globally. There are two types of DM, namely, type 2 diabetes mellitus (T2DM) and type 1 diabetes mellitus (T1DM), of which T2DM accounts for approximately 95% of all diabetes cases.[1] Individuals with T2DM frequently experience T2DM-associated complications and multiple organ damage,[2] and have a 2- to 4-fold higher risk for cardiovascular mortality compared to the general population.[3] Importantly, age is closely associated with T2DM and is an important risk factor for T2DM and cardiovascular diseases.[4] T2DM may be associated with obesity and hereditary factors, is usually accompanied by hypertension and dyslipidemia, and mainly occurs in older adults. A review study showed that aging accelerates the alteration of insulin sensitivity and is associated with impaired β-cell insulin resistance compensation, which increases insulin resistance and leads to T2DM development.[5]

DM-induced myocardial injury is one of the main complications of T2DM, and one of the causes of heart failure and death in patients with advanced diabetes.[6,7] The pathophysiological mechanisms underlying diabetic cardiomyopathy (DCM) involve cardiomyocyte apoptosis, excessive reactive oxygen species (ROS) production, overactivity of the myocardial renin-angiotensin system (RAS), and transforming growth factor beta 1 (TGF-β1) activation as well as collagen deposition, alterations in the levels of insulin-like growth factor-1 (IGF-1), and myocardial fibrosis.[8,9] However, the specific underlying mechanism has not been elucidated.

Several studies indicated that exercise training can reduce the risk for cardiovascular diseases and improve cardiac function.[10,11] A study by Dede et al. showed that exercise training improved cardiac function in male C57BL/6 mice with diabetic cardiomyopathy.[12] Another study showed that, in heart failure mice, regular moderate exercise was beneficial for improving cardiac function by increasing GLUT1 and restraining HDAC4 signaling.[13] Furthermore, exercise training, as a therapeutic intervention, has been confirmed to exert beneficial effects on the vascular system by reducing stiffness and improving endothelial function.[14] Therefore, reducing the left ventricular end-diastolic diameter and increasing the left ventricular ejection fraction (EF) benefits myocardial function by improving endothelial function.[15,16] In addition, lifestyle

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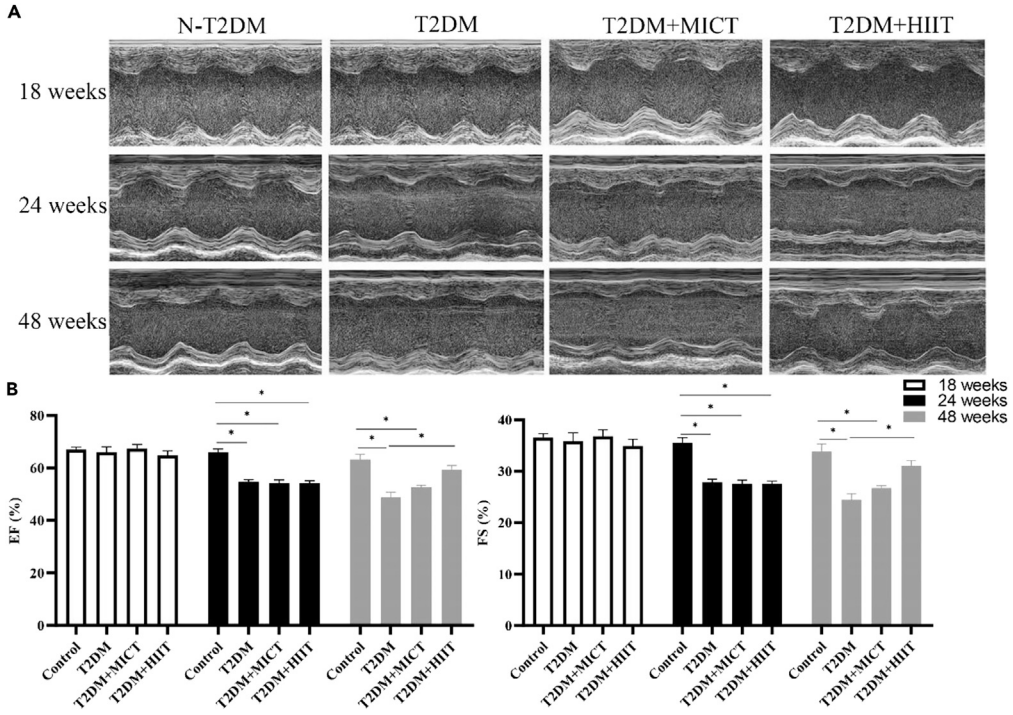

Figure 1. High-intensity interval training (HIIT) improved myocardial function in type 2 diabetes mellitus (T2DM) mice

(A) Representative M-mode echocardiogram.

(B) Left ventricular ejection fraction (EF), left ventricular FS, and heart rate were evaluated using echocardiography. n = 8, *p < 0.01.

modifications, such as exercise, can help manage T2DM. It has been demonstrated that exercise can decrease glucose levels by signaling independent of the insulin receptor.[17]

Despite the established scientific consensus that exercise is beneficial for health, particularly for patients with T2DM, little is known about their role in preventing myocardial injury in T2DM mice when moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) are initiated simultaneously. The present study aimed to determine how two different exercise training intensities, namely, MICT and HIIT influence T2DM-induced myocardial injury, and clarify the interaction between them, in a mouse model of streptozotocin (STZ)- and high-fat diet (HFD)-induced T2DM. Indeed, some experimental studies reported HIIT were more effective than MICT in reducing risks of cardiovascular disease, but other studies did not find this result. Therefore, we identified a possible mechanistic correlation between HIIT and T2DM-induced myocardial damage, providing a theoretical basis for the development of advanced and improved options to prevent and treat T2DM and its complications.

RESULTS AND DISCUSSION

HIIT improved myocardial function in T2DM mice

Parameters related to cardiac function were tested in mice to determine whether exercise training protects against cardiac injury (Figure 1A). T2DM group mice showed cardiac dysfunction, compared with control group mice. Lower fraction shortening (FS) and left ventricular ejection fraction (EF) was also observed in T2DM group mice compared with control group mice. Importantly, HIIT increased FS and EF (Figure 1B).

HIIT improved metabolic characterization

The higher level of glucose in the pre-training for week 24 experimental groups indicated that our animal models were successfully established. Exercise at any intensity reduced the glucose levels in T2DM mice,

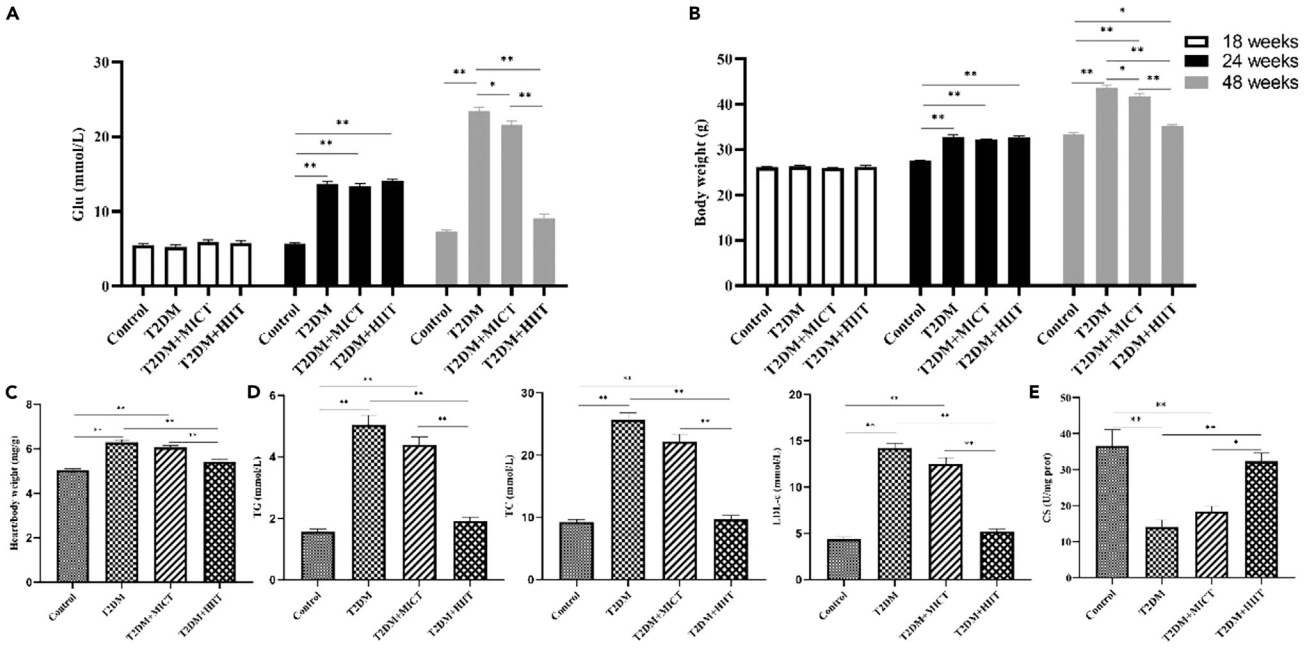

Figure 2. Metabolic data in the different groups after exercise training

(A) Quantitative analysis of the glucose (Glu) levels at baseline for week 18, pre-training for week 24, and after-training for week 48.

(B) Quantitative analysis of body weight at baseline for week 18, pre-training for week 24, and after-training for week 48.

(C) Quantitative analysis of the heart/body weight.

(D) Quantitative analysis of the triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-c) levels in the different groups. The white, black, and gray columns indicate the number of weeks (baseline for week 18, pre-training for week 24, and after-training for week 48, respectively).

(E) Mitochondrial citrate synthase activities in cardiac tissue. n = 8. *p < 0.05, **p < 0.01.

as evidenced by the lower glucose level in the two exercise groups. However, the glucose-lowering effects of HIIT were more remarkable (Figure 2A). The mice in the experimental group manifested higher body weight (after-training for week 48) and heart/body weight compared with the mice in the comparison group, but these levels were significantly reduced in the HIIT group (Figures 2B and 2C). The lipid metabolism indicators (TG, TC, and LDL-c) were prominently increased in the T2DM group but visibly decreased in the HIIT group (Figure 2D). Based on the exercise training efficacy, we further investigated the activity of citrate synthase (CS), considered a biomarker of mitochondrial abundance. The significantly decreased CS activity in the T2DM group was inhibited by both MICT and HIIT, but HIIT was superior to MICT (Figure 2E).

HIIT prevented cardiac tissue damage

To confirm the changes in cardiac tissue damage, HE, Oil Red O, Masson's trichrome, and PAS tissue-staining experiments were performed. HE staining revealed that cardiomyocytes in T2DM group exhibited breakdown, edema, disordered arrangement, twisted myocardial fibers, nuclear condensation, and increased numbers of inflammatory cells. The Masson's trichrome and PAS staining data were quantitatively analyzed (Figure 3). Masson's staining showed that there was extensive collagen deposition in the T2DM group. Obvious irregular arrangement of cardiac muscle fibers was observed, and myocardial cells were surrounded and invaded by abundant hemameba in T2DM mice, all of which were reduced by HIIT (Figure 3B). Furthermore, the areas affected by lipid accumulation, collagen deposition, and glycogen accumulation in T2DM mice were greatly decreased by HIIT (Figures 3C and 3D).

HIIT prevented cardiac hypertrophy and endothelial dysfunction

In T2DM mice, the cardiomyocyte cross-sectional area in myocardial sections was enlarged, compared with that in T2DM + HIIT mice (Figure 4A). Low levels of the endothelial cell marker CD31 were observed in T2DM mice, while high CD31 levels were observed in T2DM + HIIT mice. In T2DM mice, the expression

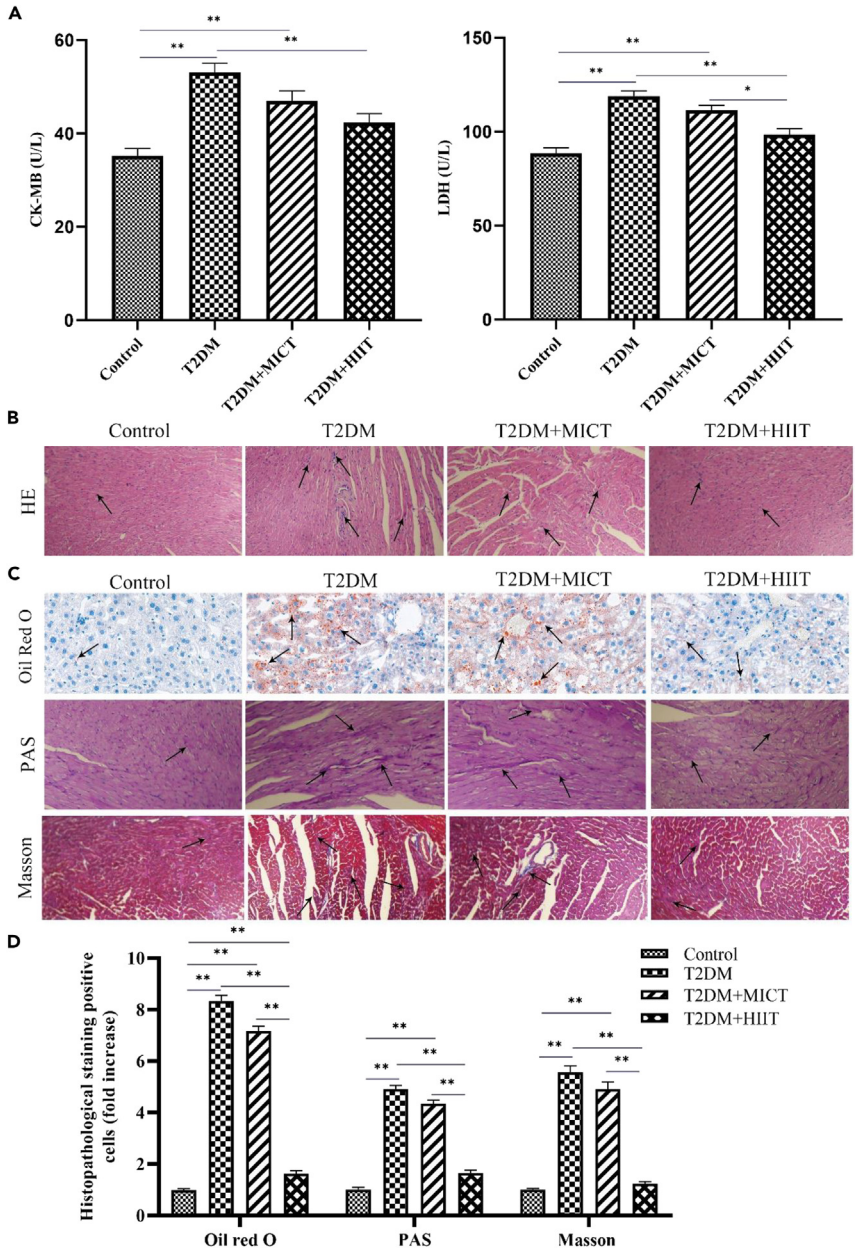

Figure 3. Cardiac tissue damage in the different groups after exercise training

(A) Quantitative analysis of the lactate dehydrogenase (LDH) and creatine kinase myocardial band (CK-MB) levels in the different groups.

(B) Hematoxylin and eosin (HE) staining showing structural damage in cardiac tissue.

(C) Oil Red O staining showing lipid accumulation in cardiac tissue. Periodic acid Schiff (PAS) staining revealing intracellular accumulation of glycogen. Masson's trichrome staining indicating myocardial fibrosis.

(D) Quantitative analysis of the intracellular accumulation of glycogen and myocardial fibrosis. Magnification 40×. The arrows indicate the positively stained cells. n = 3. *$p < 0.05$, **$p < 0.01$.

of sirtuin-3 (SIRT3) was low, nonetheless, this low expression tendency was prevented by HIIT. Interestingly, CD31 and SIRT3 were colocalized (Figures 4B and 4C).

To further evaluate the endothelial function, related indicators were tested. Increased intercellular adhesion molecule-1 (Icam-1) and endothelin-1 (ET-1) levels and decreased endothelial nitric oxide synthase

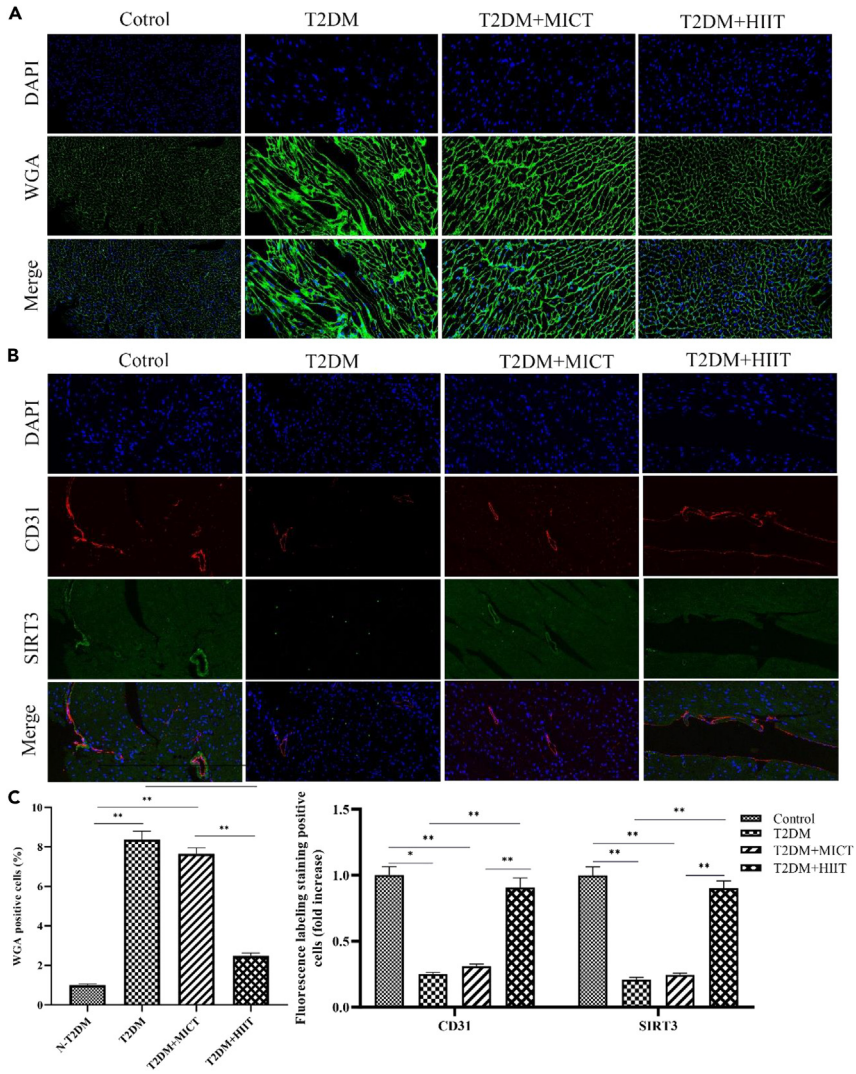

Figure 4. HIIT prevented cardiac hypertrophy and endothelial dysfunction

(A) Representative photomicrographs of the myocardium stained with wheat germ agglutinin (WGA, green fluorescence) and 4′,6-diamidino-2-phenylindole (DAPI, blue fluorescence).

(B) Representative photomicrographs of the myocardium stained for CD31 (red fluorescence), sirtuin-3 (SIRT3, green fluorescence), and DAPI (blue fluorescence).

(C) Quantitative analysis of WGA, SIRT3 and CD31 expression. Magnification 40×. The arrows indicate the positively stained cells. n = 3. *$p < 0.05$, **$p < 0.01$.

(eNOS) levels were observed in the T2DM group. In contrast, the opposite tendencies were observed in the T2DM + HIIT group (Figure 5).

HIIT prevented myocardial fibrosis in T2DM mice

Metrics, such as collagen I (Co I), collagen III (Co III), Smad3, and TGF-β, associated with myocardial fibrosis were tested. The expression levels of Co I, Co III, Smad3, and TGF-β were increased in the T2DM group, while they were decreased in the T2DM + MICT and T2DM + HIIT groups, particularly in the T2DM + HIIT group (Figure 6).

HIIT prevented oxidative stress in T2DM mice

The cardiac GSH level was significantly decreased, and the MDA level was increased in T2DM mice compared with those in control mice. Nonetheless, HIIT attenuated the MDA level and restored the

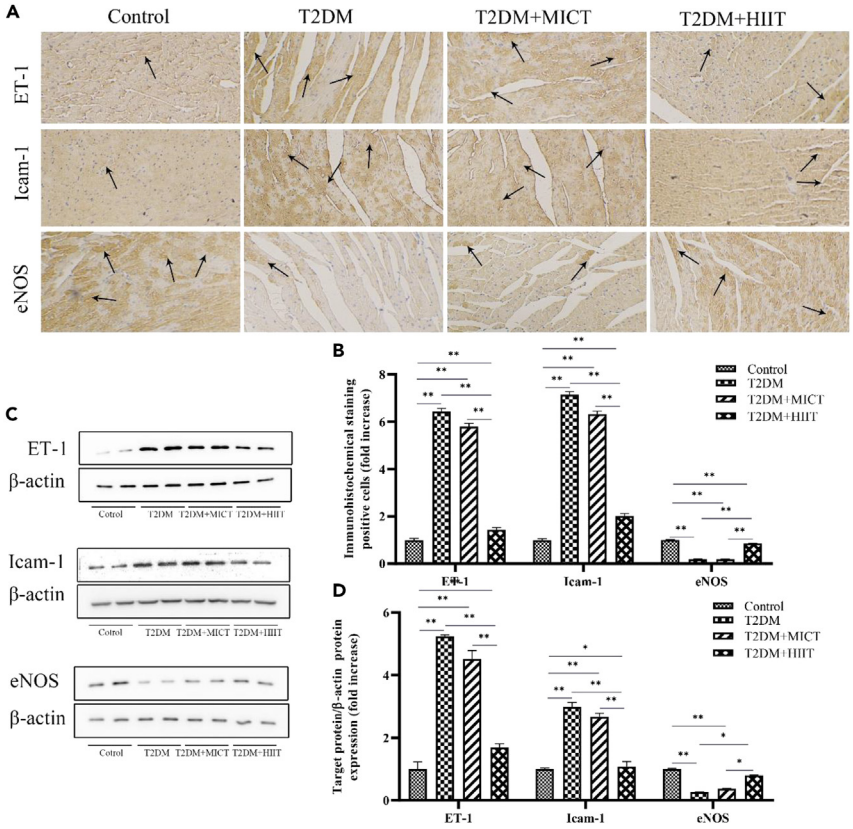

Figure 5. Endothelial function in the different groups after exercise training

(A) Representative immunohistochemistry for endothelin-1 (ET-1), intercellular adhesion molecule-1 (Icam-1), and endothelial nitric oxide synthase (eNOS) in cardiac tissues. Magnification 40×.

(B) Quantification of relative protein expression levels.

(C) ET-1, Icam-1, and eNOS levels assessed via Western blotting. The original gels are displayed in the supplementary file.

(D) Quantification of ET-1, Icam-1, and eNOS levels via Western blotting. The arrows imply positively stained cells. n = 3. *p < 0.05, **p < 0.01.

GSH level (Figure 7A). A dihydroethidium (DHE) fluorescent probe was used to evaluate the cardiac ROS levels. The ROS level in the heart was reduced following HIIT treatment compared with that in the T2DM group (Figures 7B and 7C), suggesting that HIIT reduced cardiac injury by reducing ROS accumulation. Furthermore, immunohistochemistry (Figures 7D and 7E) was used to detect the levels of oxidative stress indicators. The levels of NRF2, HO-1, and SOD, which were significantly downregulated in the T2DM mouse heart, were restored by HIIT. Furthermore, compared with control mice, the expression of NOX4 was increased in T2DM mice and decreased by HIIT.

HIIT prevented apoptosis in T2DM mice

The number of TUNEL-positive cells was increased in the heart of T2DM mice compared with that in the heart of T2DM + HIIT mice, whereas cardiac apoptosis was reduced in T2DM + HIIT mice (Figures 8A and 8B). Furthermore, the immunohistochemistry and Western blotting results showed that in the T2DM group, the expression of the pro-apoptotic proteins Bcl-2 decreased, while that of BAX and Bak increased. However, HIIT treatment increased the expression of the pro-apoptotic protein Bcl-2 and decreased that of BAX and Bak (Figures 8C–8F). Thus, HIIT exerted beneficial effects on cardiac function in T2DM mice.

MICT and HIIT decreased the levels of natural aging markers in T2DM mice

The levels of heat shock factor 1 (HSF1) were reduced in T2DM mice. However, both MICT and HIIT increased the expression level of HSF1, especially HIIT (Figures 9A and 9B).

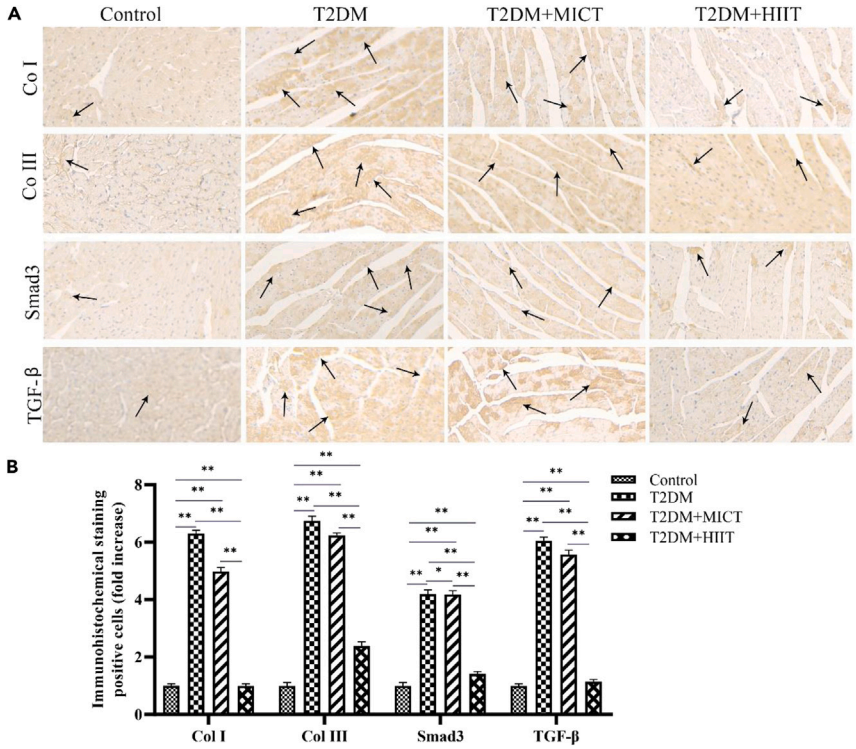

Figure 6. Myocardial fibrosis in the different groups after exercise training

(A) Representative immunohistochemistry for collagen I (Co I), collagen III (Co III), Smad3, and transforming growth factor-β (TGF-β) in cardiac tissues.

(B) Quantification of the relative protein expression levels. Magnification 40×. The arrows indicate the positively stained cells. n = 3. *p < 0.05, **p < 0.01.

DISCUSSION

This study in male C57BL/6J mice showed that HIIT, rather than MICT, for 24 weeks can alleviate T2DM-associated cardiac dysfunction. T2DM-associated cardiac dysfunction involves processes such as myocardial remodeling, myocardial fibrosis, oxidative stress, and apoptosis. HIIT alleviates cardiac dysfunction and restores cardiac damage, whereas the beneficial effects of MICT on cardiac function are less prominent. These findings are presented in Figure 10.

Exercise training benefits cardiac function and improves quality of life in patients with cardiac dysfunction and healthy individuals.[18] In the study, T2DM group mice showed cardiac dysfunction, and HIIT significantly increased FS and EF. Furthermore, cardiovascular disease risks can be reduced via molecular, cellular, and metabolic adaptations and oxidative stress in health and disease.[19–21] Baekkerud et al. detected mitochondrial dysfunction in the heart of T2DM mice, which was partly ameliorated by exercise training.[22] Despite the beneficial effects of exercise on cardiac function in patients with T2DM, the impact of optimal exercise training intensity on these patients has not been studied.

A previous study indicated that T2DM can induce vascular damage.[23,24] CS is one of the first enzymes participating in the citric acid cycle and cellular oxidative metabolism. This shows that T2DM may interfere with the transcriptional programming of cardiac mitochondria; the mitochondrial content was significantly reduced in T2DM mice, and was significantly increased in HIIT mice, which confirmed that HIIT was superior to MICT. Dyslipidemia is considered a T2DM-related risk factor that promotes the formation of atherosclerotic plaques via promoting platelet hyperactivation, inflammation, and endothelial dysfunction.[25–27] Diabetic dyslipidemia is generally connected to insulin resistance and is characterized by increases in LDL-c, TG, and TC levels.[28] In our study, the body weights of exercise training mice were lower than that of T2DM mice. Moreover, HIIT prevented these T2DM-related injuries and improved metabolic characteristics.

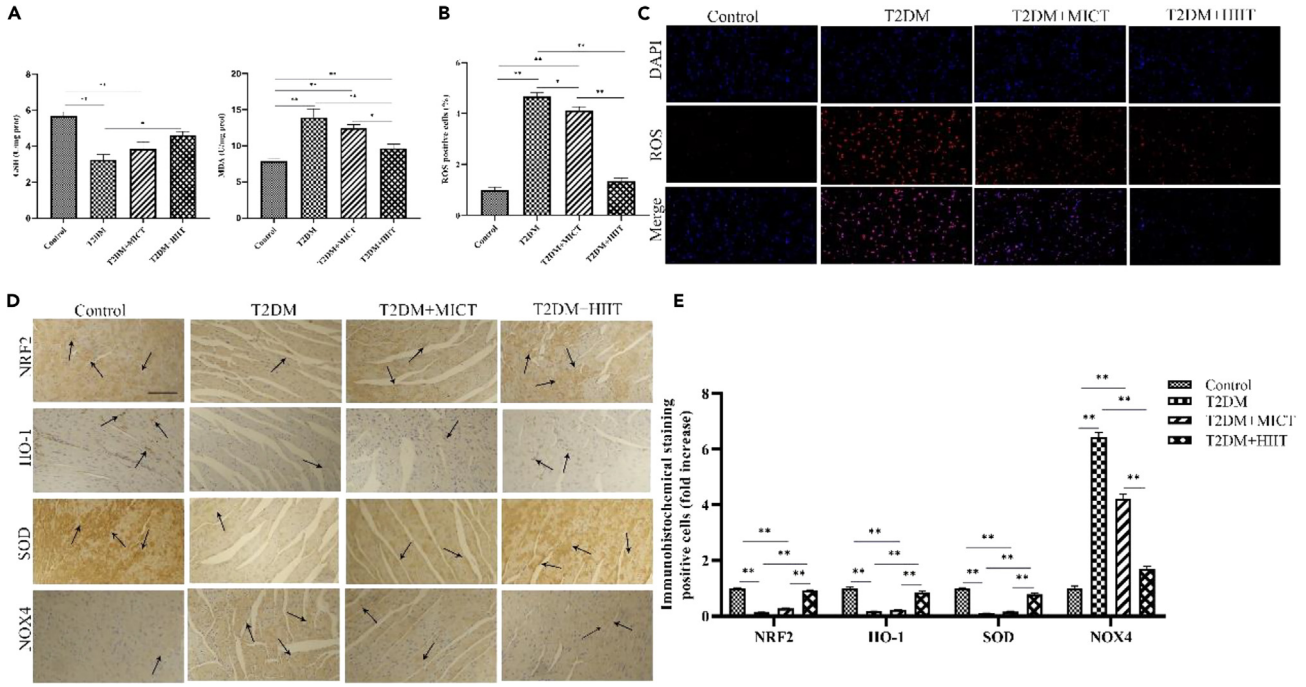

Figure 7. High-intensity interval training (HIIT) decreased oxidative stress in type 2 diabetes mellitus (T2DM) mice

(A) Cardiac malondialdehyde (MDA) and glutathione (GSH) levels quantified using commercial assay kits. n = 8.

(B) Quantitative analysis of reactive oxygen species (ROS) levels.

(C) ROS analysis.

(D) Representative immunohistochemistry images showing the expression of nuclear factor erythroid 2–related factor 2 (NRF2), heme oxygenase-1 (HO-1), superoxide dismutase (SOD), and NADPH oxidase 4 (NOX4) in cardiac tissues.

(E) Quantification of the relative protein expression levels. Magnification 40×. The arrows indicate the positively stained cells. n = 3. *$p < 0.05$, **$p < 0.01$.

Diabetic cardiomyopathy may affect individuals with T2DM. It typically manifests first as left ventricular hypertrophy and diastolic dysfunction.[29,30] Myocardial remodeling, myocardial hypertrophy, and fibrosis occur in the T2DM hearts.[31] A study showed that the prevalence of ventricular remodeling and hypertrophy was elevated in both individuals with prediabetes and T2DM compared with that in healthy individuals.[32] According to Jia G et al., the early stages of diabetic cardiomyopathy progression are usually asymptomatic and characterized by subclinical functional and structural transformations including reduced LV compliance and increased left ventricular hypertrophy and myocardial fibrosis.[33] Studies have shown that activation of the TGF-β1/Smad3 pathways in cardiac fibrosis can lead to myofibroblast proliferation and a significant upregulation in the collagen I expression levels.[34] Furthermore, a review highlighted the potential of Co I and Co III as myocardial fibrosis indicators.[35] Our study showed that T2DM caused cardiac remodeling and fibrosis. Furthermore, HIIT significantly alleviated myocardial remodeling and fibrosis, compared with MICT.

SIRT3 is found in the nucleus, mitochondrial matrix, and cytoplasm.[36–39] In our study, the expression level of SIRT3 was increased by HIIT compared with that in T2DM (Figures 4B and 4C). Recent reports showed that SIRT3 is involved in the regulation of mitochondrial function[40] and protection against several heart diseases and that it protects cardiomyocytes from oxidative stress-mediated cellular damage.[41] Importantly, a previous study emphasized that compared with WT mice, SIRT3 KO mice showed worse neurobehavioral outcomes accompanied by decreased angiogenesis due to reduced CD31 expression in the perifocal region during recovery after ischemic stroke.[42] Furthermore, SIRT3 deficiency reduced the activation of vascular endothelial growth factor, AKT, and extracellular signal-regulated kinase signaling. Therefore, we speculated that SIRT3 may promote CD31 expression to antagonize the cell stress response. Fortunately, the relevant double immunofluorescence staining results were consistent with this speculation. Endothelial cells are more susceptible than other cell types to hyperglycemia induced damage, and endothelial dysfunction may contribute to diabetic cardiomyopathy.[43] Furthermore, a review illustrated that Icam-1 transduced outside-in signaling and connected leukocyte adhesive interactions with epithelial and

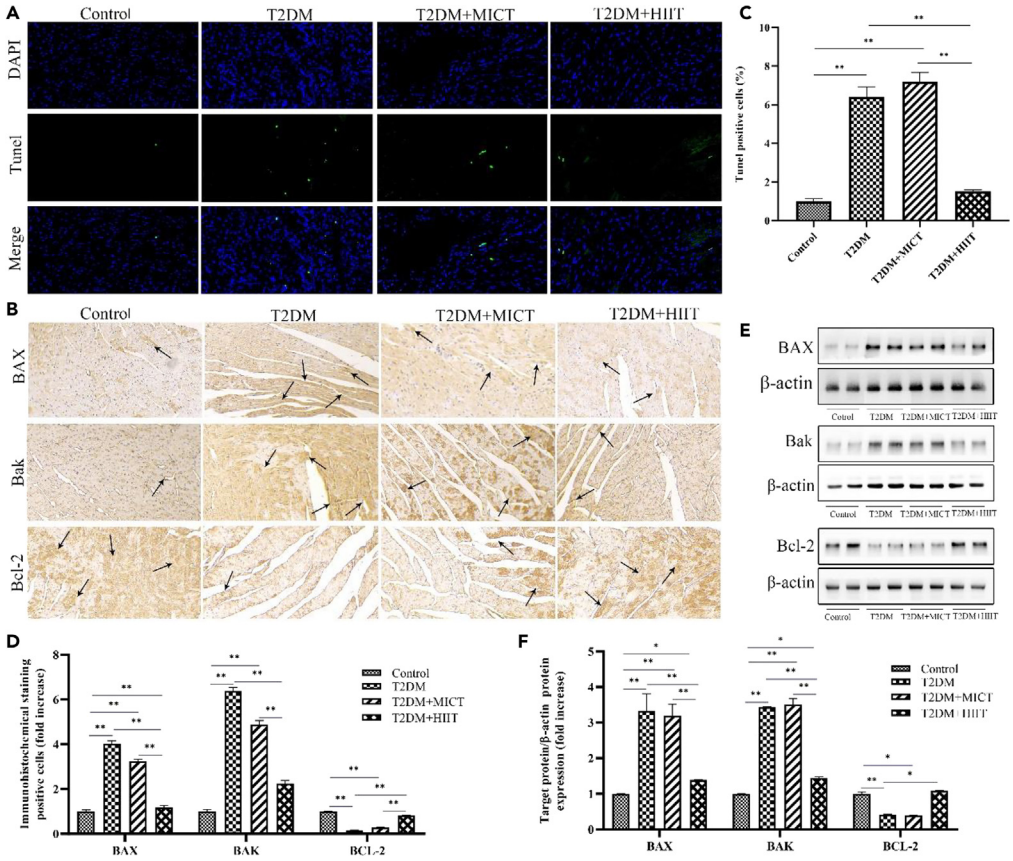

Figure 8. High-intensity interval training (HIIT) attenuated apoptosis in type 2 diabetes mellitus (T2DM) mice

(A) TUNEL-stained (green fluorescence) and DAPI-stained (blue fluorescence) photomicrographs. Magnification 40×.

(B) Representative immunohistochemistry images showing the expression of cardiac BAX, Bcl-2, and Bak in cardiac tissues. Magnification 40×. The arrows indicate the positively stained cells.

(C) Quantitative analysis of apoptotic cardiomyocytes, n = 3.

(D) Quantification of the BAX, Bak, and Bcl-2 expression levels.

(E) BAX, Bak, and Bcl-2 levels assessed using Western blotting. The original gels is displayed in the supplementary file.

(F) Quantification of BAX, Bak, and Bcl-2 levels assessed using Western blotting. n = 3. *p < 0.05, **p < 0.01.

endothelial function.[44] L Madden et al. showed that endothelial microvesicles generated by ET-1 affected endothelial cell apoptosis, inflammation, and eNOS.[45]

Thomas et al. revealed that ROS negatively affect myocardial calcium handling, induce hypertrophy and apoptosis, and, as a result, lead to cardiac remodeling.[46] Our study demonstrated that, compared with MICT, the index of ROS significantly decreased by HIIT in T2DM (Figures 5B and 5C). Furthermore, HIIT reduced the levels of metabolites and oxidant proteins, such as NOX4, and increased the levels of antioxidant proteins, such as HO-1, SOD, and GSH. According to previous studies, a major function of NRF2 is to resist oxidant stress.[47] Bellezza et al. reported that NRF2 is a potential target in disease prevention since its antioxidant effects lead to cell protection.[48] In this study, HIIT, rather than MICT, upregulated the expression of NRF2 in myocardial tissues.

Many apoptotic cellular processes are associated with mitochondria. Mitochondrial dysfunction generally arises in patients with T2DM[49] and threatens organ systems.[50,51] Moreover, Jia et al. regarded movement training as a lifestyle factor that protects against cardiac apoptosis.[19] In our study, apoptosis was mainly decreased by HIIT.

There is an inseparable relationship between aging and cell senescence. A study indicated that the incidences and prevalence of T2DM and impaired glucose tolerance (IGT) are higher in older adults compared

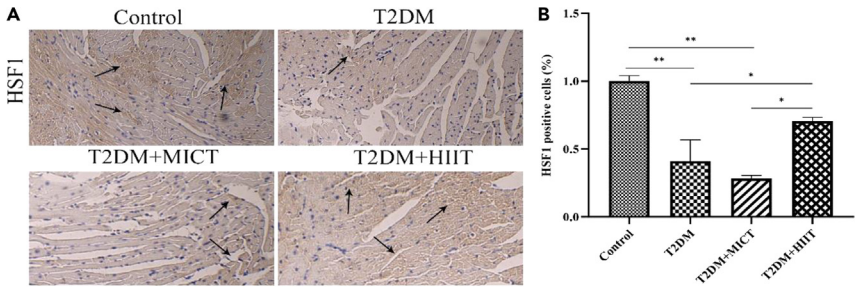

Figure 9. High-intensity interval training (HIIT) decreased the levels of natural aging markers in type 2 diabetes mellitus (T2DM) mice

(A) Representative immunohistochemistry for HSF1. Magnification 40×.

(B) Quantification of the HSF1 expression levels. n = 3. **p < 0.01.

with those in younger individuals.[52] However, the mechanism connected with the glucose intolerance level in older adults is still not elucidated. Importantly, there is no specific treatment for age-induced damage at the moment. In T2DM mice, the index of cell senescence vastly increased, implying that T2DM may promote aging. In our study, MICT and HIIT decreased the levels of natural aging markers in T2DM mice. Moreover, HIIT was overall more efficient than MICT.

Limitations of the study

This study has some limitations. Firstly, all mice selected for this study are male. Thus, the influences caused by the distinct hormone levels between male and female mice were not studied. In addition, the sample size was small. Importantly, these findings need to be verified in patients.

The research only male mice were used; exercise cycle in this study was 24 weeks, and if the exercise cycle is extended, both exercise intensities may be benefit; there is little research on how exercise can alleviate the phenotype of T2DM.

In summary, this study clearly indicates the protective effect of HIIT on myocardial injury in T2DM mice by increasing cardiac function and decreasing myocardial remodeling, myocardial fibrosis, oxidative stress,

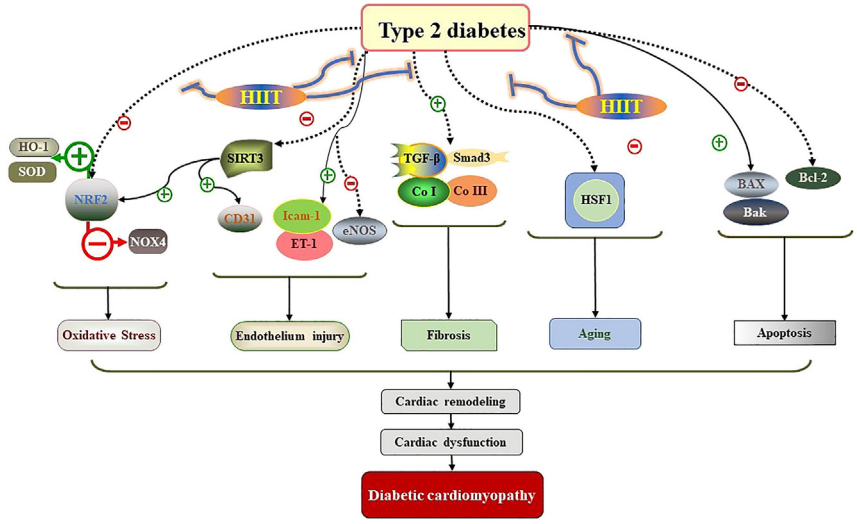

Figure 10. Schematic diagram showing how high-intensity interval training (HIIT) protects against cardiac damage in type 2 diabetes mellitus (T2DM) mice

HIIT can reduce myocardial remodeling, oxidative stress, endothelium injury, apoptosis, and the aging-related marker levels to prevent dysfunction and damage in the T2DM heart.

and apoptosis. According to our results, HIIT may be a crucial treatment to prevent T2DM-induced cardiac dysfunction. However, clinical experiments are required to determine if HIIT can alleviate the myocardial injury caused by T2DM.

Conclusion

T2DM is usually accompanied by cardiac dysfunction and myocardial damage, which severely affect health and daily life. Fortunately, our findings suggest that HIIT may be an effective means to guard against T2DM-induced myocardial injury.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107080.

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AUTHOR CONTRIBUTIONS

Conceptualization, supervision and project administration, Z. P.; Writing – original draft and software, R. Z.; Investigation and data curation, W. Y.; Formal analysis, S. D.; Methodology, Y. L.; Funding acquisition, Z. G. and Z. P.. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
rabbit anti-NOX4	Proteintech	Cat# No. 14347-1-AP; RRID: AB_10638146
rabbit anti-NRF2	Proteintech	Cat# No. 80593-1-RR; RRID: AB_2918904
rabbit anti-HO-1	Proteintech	Cat# No. 10701-1-AP; RRID: AB_2118685
rabbit anti-SOD	Proteintech	Cat# No. 10269-1-AP; RRID: AB_2193750
rabbit anti-Icam-1	Proteintech	Cat# No. 10831-1-AP; RRID: AB_2264494
rabbit anti-ET-1	Proteintech	Cat# No. 12191-1-AP; RRID: AB_889392
rabbit anti-eNOS	Proteintech	Cat# No. 27120-1-AP; RRID: AB_2880764
rabbit anti-TGF-β	Proteintech	Cat# No. 21898-1-AP; RRID: AB_2811115
rabbit anti-Smad3	Proteintech	Cat# No. 61249; RRID: AB_2793568;
rabbit anti-Co I	Proteintech	Cat# No. 14695-1-AP; RRID: AB_2082037
rabbit anti-Co III	Proteintech	Cat# No. 22734-1-AP; RRID: AB_2879158
rabbit anti-BAX	Proteintech	Cat# No. 50599-2-Ig; RRID: AB 2061561
rabbit anti-Bak	Proteintech	Cat# No. 29552-1-AP
rabbit anti-Bcl-2	Proteintech	Cat# No. 26593-1-AP; RRID: AB_2818996
rabbit anti-β-actin	Proteintech	Cat# No. 81115-1-RR
HRP-conjugated Affinipure Goat Anti-Rabbit IgG(H+L)	Proteintech	Cat# No. SA00001-2; RRID: AB_2722564
Critical commercial assays		
Total cholesterol assay kit	Nanjing Jiancheng Bioengineering Institute	Cat# A111-1
Low-density lipoprotein cholesterol assay kit	Nanjing Jiancheng Bioengineering Institute	Cat# A113-1
Triglyceride assay kit	Nanjing Jiancheng Bioengineering Institute	Cat# A110-1
lactate dehydrogenase assay kit	Nanjing Jiancheng Bioengineering Institute	Cat# A020-2
creatine kinase myocardial band assay kit	Nanjing Jiancheng Bioengineering Institute	Cat# E006
Citrate Synthase Activity Assay Kit	Solarbio	Cat# BC1060
Fluorescein TUNEL Cell Apoptosis Detection kit	Servicebio	Cat# T2190
Experimental models: Organisms/strains		
Mice: C57BL/6JSmoc	Shanghai Model Organisms Center	Cat# No. SM-001
Software and algorithms		
SPSS software version 23.0	SPSS software version 23.0	N/A
Other		
Treadmill	Shanghai XinRuan Information Technology Co., Ltd.	No. XR-PT-10B
Vevo 2100LT micro-ultrasound system	FUJIFILM VisualSonics, Inc.	N/A

RESOURCE AVAILABILITY

Lead contact

Requests for resources and reagents should be directed to the lead contact Zuowei Pei (pzw_dl@163.com).

Materials availability

All reagents and materials will be made available on request after completion of a Materials Transfer Agreemen.

Data and code availability

This study did not generate original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request. All data produced in this study are included in the published article and its supplemental information, or are available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animals

Thirty-two 17-week-old male C57BL/6J mice were purchased from Shanghai Model Organisms Center (Shanghai, China) and allowed to adapt to the new environment for one week. Subsequently, mice were randomly divided into four groups (n = 8 per group): a control group, and three experimental groups, including the T2DM, T2DM + medium-intensity continuous training (T2DM+MICT), and T2DM + high-intensity interval training (T2DM+HIIT) groups. To establish the T2DM model, 50 mg/kg streptozotocin (sigma, Germany) was injected into the enterocoelia of mice in the experimental groups, and mice were fed a HFD (fat 60%, protein 20%, carbohydrate 20%, Greisway Biotechnology Co., Ltd. Suzhou, China).[53] Experimental group mice were fed high-fat foods for six weeks. Subsequently, mice were divided into the two exercise training groups and subjected to exercise five days per week for 24 consecutive weeks. Four mice were housed per cage, and all the mice were housed in a temperature-controlled room with free access to tap water and food with a 12h/12h-light/dark cycle.

At the end of the experiments, the mice were administered a lethal dose of pentobarbital (40 mg/kg, intraperitoneally). Blood was, then, collected from the eye sockets of the mice and allowed to clot. The clotted blood was centrifuged to obtain serum and the serum samples were stored at −80°C. Then, mice were decapitated and confirmed to be dead when cessation of heartbeat and respiration was observed. The cardiac tissue was frozen in liquid nitrogen for mRNA isolation and immunoblotting analyses. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of the Dalian Municipal Central Hospital.

Exercise training regimen

Mice in the two exercise training groups were subjected to exercise five days per week for 24 consecutive weeks before blood and heart tissue were collected. Every afternoon, the mice were subjected to the following training regimens on a treadmill (No. XR-PT-10B; Shanghai XinRuan Information Technology Co., Ltd. Shanghai, China). The maximal exercise of mice was tested every Saturday to determine the running speed of different groups of mice for the following week. Specific exercise doses are described below: HIIT exercises contained 9 sets of 1.5 min high-intensity running [85% maximum velocity (Vmax)] sessions, with 1 min of active rest time in between sets and MICT exercises contained in 60% Vmax. The same movement distance should be kept in HIIT and MICT.[54] One week before training, the mice were allowed to adapt to the treadmill. All mice were in similar post-exercise condition. The T2DM group mice were placed on the track of the treadmill but their movements were not controlled by human intervention.

Echocardiography

The Vevo 2100LT micro-ultrasound system (FUJIFILM VisualSonics, Inc., Ontario, Canada) was used to perform an Ultrasonic test in animals after treatment. First, the mice were anesthetized with 1.5% isoflurane, and then rapidly placed on a heating pad heated to 37°C to keep the normal body temperature. The direction of the ultrasound beam needed to be slowly adjusted to obtain an echocardiogram of the left ventricle. Left ventricular function parameters were evaluated via M-mode images. The M curve was measured at the long axis of papillary muscle and left ventricle section level. The following variables were measured and averaged during 3 consecutive cardiac cycles: left ventricular end systolic diameter (LVESD), left ventricular end diastolic diameter (LVEDD), left ventricular end systolic volume (LVSV) and left ventricular end diastolic volume (LVDV). The left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) values were converted by the Simpson method with the following formula: LVEF = (LVDV − LVSV)/LVDV × 100%; LVFS = (LVDD − LVSD)/LVDD × 100%. LVEF and LVFS were used as parameters indicating cardiac function. The experiment was conducted 3 times, and the mean value was obtained.

Serum measurements

The serum was separated from the collected blood samples using centrifugation at 3000 rpm for 15 min. The serum levels of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-c), and triglyceride (TG) were detected using an assay kit, according to the manufacturer's instructions. The serum lactate dehydrogenase (LDH) and creatine kinase myocardial band (CK-MB) levels were examined using commercial reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Measurement of citrate synthase activity

To measure the activity of CS activity, the heart tissue of mice was weighed and placed in an ice-cold 20 mmol/L Tris hydrochloride buffer (pH 7.8) containing 0.2% Triton X-100 and protease inhibitor cocktail. The tissue-to-buffer ratio was 1:20 (w:v). The heart was finely cut with scissors and then homogenized. The obtained homogenate was centrifuged at 13,000× g for 10 min at 4°C. The supernatant was collected and determined by measuring coenzyme A formation at 412 nm with Citrate Synthase Activity Assay Kit (Solarbio BC 1060, China) according to the manufacturer's instruction.

Histological staining

Cardiac tissues were stored in 10% formalin, dehydrated in an ascending alcohol series (75, 85, 90, and 100% alcohol, 5 min each), and then embedded in paraffin wax. Paraffin-embedded tissue blocks were sliced into 4 μm thick sections, which were then de-paraffinized via immersion in xylene (thrice, 5 min each) and rehydrated using a descending alcohol series (100, 90, 85, and 75% alcohol, 5 min each). Histological changes were detected by staining the sections with hematoxylin and eosin (HE), Masson's trichrome, Periodic Acid-Schiff (PAS), and FITC-conjugated wheat germ agglutinin (WGA) stain. Images were acquired using an upright light microscope (Olympus, Tokyo, Japan).

Cardiac oxidative stress analysis

Cardiac tissue was homogenized in saline solution at a ratio of 1:9 mg/μL. After the homogenate was spun for 5 min at 7,000 rpm, the supernatant was collected and used for glutathione (GSH) and malondialdehyde (MDA) measurements using commercially available kits according to the manufacturer's directions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

ROS analysis

The heart tissues were embedded in an embedding agent at the optimal cutting temperature. Next, 6-μm-thick sections were prepared using a freezing microtome for ROS staining. At room temperature, the frozen sections were fixed with 4% PFA for 5 min, rinsed thrice with PBS (1 min each time), and then a tissue pen was used to draw a circle around the sections followed by the addition of 5 μM 20,70-dichlorofluorescein diacetate (DCFH-DA) to the circled areas. Sections were incubated for 20 min at 37°C in the dark, followed by PBS washes and DAPI staining for 10 min at room temperature. Next, each section was washed thrice with PBS (5 min each time). The sections were sealed with an antifluorescence quenching sealant and observed and imaged using a camera-equipped fluorescence microscope.

Oil red O staining

The 6-μm-thick frozen sections were stained with Oil red O stain solution for 10 min, immersed in two cups of 60% isopropanol for differentiation in turn, for 3 s and 5 s, respectively, and then stained with hematoxylin for 5 min. Finally, sections were rinsed in three cups of pure water for 5 s, 10 s, and 30 s in turn, treated with differentiation solution (60% alcohol as solvent) for 5 s, then rinsed in two cups of distilled water for 10 s each, and in Scott Tap Bluing for 1 s, prior to observation and evaluation under a microscope. The lipid contents in the sections of the cardiac tissues were assessed by oil red O staining.

Immunohistochemistry

Coronal sections of heart tissues were fixed in 10% formalin, dehydrated in an ascending series of ethanol, and embedded in paraffin for histological evaluation. Sections were deparaffinized and rehydrated. Next, they were blocked with 3% H2O2 in methanol for 15 min to deactivate endogenous peroxidases and then cultivated overnight at 4°C with one of the following primary antibodies: rabbit anti-NOX4, (1:100; Proteintech), rabbit anti-NRF2 (1:200; Proteintech), rabbit anti-HO-1 (1:100; Abcam, Cambridge, UK), rabbit anti-SOD (1:200; Proteintech), rabbit anti- Icam-1 (1:100; Proteintech), rabbit anti-ET-1 (1:100; Proteintech), rabbit anti-eNOS (1:200; Proteintech), rabbit anti-TGF-β (1:200; Proteintech), rabbit

anti-Smad3 (1:100; Proteintech), rabbit anti-Co I (1:500; Proteintech) and rabbit anti-Co III (1:1000; Proteintech). The sections were then incubated for 30 min at room temperature with a goat anti-rabbit HRP secondary antibody (Anti-rabbit Universal Immunohistochemical Detection Kit; Proteintech). All sections were examined using an Olympus B×40 upright light microscope (Olympus, Tokyo, Japan).

TUNEL staining

The hearts were embedded in paraffin and serially sliced into 5 μm-thick sections. Sections were deparaffinized and hydrated in xylene and gradient concentrations of ethanol, and then incubated in proteinase K (37°C, 22 min) and stained using the Fluorescein TUNEL Cell Apoptosis Detection kit (Servicebio Technology Co., Ltd., Wuhan, China). All images were captured using a fluorescence microscope (Nikon). The cells that were positive for TUNEL staining and aligned with DAPI staining were considered apoptotic and counted.

Western blotting

Proteins were extracted using radioimmunoprecipitation assay buffer (P0013B; Beyotime, Shanghai, China). The protein samples were first separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to polyvinylidene fluoride membranes (Immobilon, Millipore, Billerica, MA, USA). The membranes were blocked with 5% skim milk in TBST buffer (TBS containing 0.1% Tween-20) at room temperature for 1 h, and then incubated with one of the following primary antibodies at 4°C overnight: rabbit anti-BAX (1:5000; Proteintech), rabbit anti-Bak (1:1000; Proteintech), rabbit anti-Bcl-2 (1:2000; Proteintech), and rabbit anti-β-actin (1:1000; Proteintech). After washing, the membranes were incubated with the appropriate secondary antibody (anti-rabbit Ig-G, 1:2000; Proteintech) for 1 h. The immunoreactive proteins were quantified using the NIH ImageJ software. β-Actin was used as an internal control. The protein levels are expressed as protein/β-actin ratios.

QUANTIFICATION AND STATISTICAL ANALYSIS

All data are expressed as the mean ± SEM. All analyses were performed using the SPSS software version 23.0. Differences among multiple groups were examined using one-way ANOVA followed by Tukey's test. A P-value of < 0.05 was considered statistically significant.