Aerobic Exercise Inhibited P2X7 Purinergic Receptors to Improve Cardiac Remodeling in Mice with Type 2 Diabetes

Ting Wang  
Wenzhou Medical University First Affiliated Hospital Department of Cardiology

Jianmin Li  
Wenzhou Medical College First Affiliated Hospital: The First Affiliated Hospital of Wenzhou Medical University

Hui Li  
The First Affiliated Hospital of Wenzhou Medical University

Xin Zhong  
The First Affiliated Hospital of Wenzhou Medical University Department of Cardiology

Luya Wang  
Wenzhou Medical University First Affiliated Hospital Department of Cardiology

Shuyu Zhao  
The First Affiliated Hospital of Wenzhou Medical University

Xuesheng Liu  
Wenzhou Medical University First Affiliated Hospital Department of Cardiology

Zhouqing Huang (✉ susiehzq@126.com)  
The Key of Laboratory of Cardiovascular Disease of Wenzhou, Department of Cardiology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China  https://orcid.org/0000-0001-5854-1377

Yonghua Wang  
Wenzhou Medical University

Original investigation

Keywords: Aerobic exercise, P2X7 purinergic receptors, Diabetic cardiomyopathy, Cardiac remodeling

Posted Date: November 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-978243/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

Background

Diabetic cardiomyopathy (DCM), the main complication of diabetes mellitus, presents as cardiac dysfunction by ventricular remodeling. In addition, the inhibition of P2X7 purinergic receptors (P2X7R) alleviates cardiac fibrosis and apoptosis in Type 1 diabetes. However, whether exercise training improves cardiac remodeling by regulating P2X7R remains unknown.

Methods

Db/db mice spontaneously induced with type 2 diabetes and high-fat diet (HFD) and mice with streptozotin (STZ)-induced type 2 diabetes mice were treated by 12-week treadmill training. Cardiac functions were observed by two-dimensional echocardiography. Hematoxylin-eosin staining, Sirius red staining and transmission electron microscopy were respectively used to detect cardiac morphology, fibrosis and mitochondria. In addition, real-time polymerase chain reaction and Western Blot were used to detect mRNA and protein levels.

Results

Studying the hearts of db/db mice and STZ-induced mice, we found that collagen deposition and the number of disordered cells significantly increased compared with the control group. However, exercise markedly reversed these changes, and the same tendency was observed in the expression of MMP9, COL-I, and TGF-β, which indicated cardiac fibrotic and hypertrophic markers, including ANP and MyHC expression. In addition, the increased Caspase-3 level and the ratio of Bax/Bcl2 were reduced by exercise training, and similar results were observed in the TUNEL test. Notably, the expression of P2X7R was greatly upregulated in the hearts of db/db mice and HFD+STZ-induced DM mice and downregulated by aerobic exercise. Moreover, we indicated that P2X7R knock out significantly reduced the collagen deposition and disordered cells in the DM group. Furthermore, the apoptosis levels and TUNEL analysis were greatly inhibited by exercise or in the P2X7R−/− group in DM. We found significant differences between the P2X7R−/−+DM+EX group and DM+EX group in myocardial tissue apoptosis and fibrosis, in which the former is significantly milder. Moreover, compared with the P2X7R−/−+DM group, the P2X7R−/−+DM+EX group represented a lower level of cardiac fibrosis. The expression levels of TGF-β at the protein level and TGF-β and ANP at the genetic level were evidently decreased in the P2X7R−/−+DM+EX group.

Conclusion

Aerobic exercise reversed cardiac remodeling in diabetic mice at least partly through inhibiting P2X7R expression in cardiomyocytes.

Introduction

In recent years, the global prevalence of diabetes, particularly type 2 diabetes mellitus (T2DM), has been increasing in a disturbing manner[1–3]. Diabetic cardiomyopathy (DCM) is a comorbidity of diabetes mellitus, characterized by impairment of the myocardial structure and functional damage in the absence of coronary atherosclerosis, valvular disease, and overt clinical coronary artery disease (CAD)[4, 5]. In addition, DCM is manifested in cardiac enlargement,
hypertrophy, myocardial lipid accumulation, fibrosis, and cardiac dysfunction[6]. For the underlying mechanism of DCM, cells oxidation, excessive production of reactive oxygen species (ROS), and endoplasmic reticulum stress are thought to be drivers of cell death, including apoptosis and autophagy, which contribute to subsequent replacement fibrosis, followed by deterioration of heart function[7–10].

P2X7R, a neuronal P2X receptor, is an ATP-gated cationic channel composed of three subunits[11]. Considerable evidence has shown that P2X7R is involved in the regulation of multiple disease progression. For example, the inhibition of P2X7R restores dendritic spine dynamics and relieves social behavioral deficits in mice with Rett syndrome[12], leading to the reduction of inflammation[13, 14], hepatocyte apoptosis[15, 16], and ureteral obstructive reaction collagen deposition[17]. In recent years, the role of P2X7R in cardiovascular disease has received considerable attention[18, 19], with the expression of P2X7R in coronary artery and myocardial tissue becoming a consensus. Studies have revealed that P2X7R is involved in the progression of atherosclerosis[20, 21]. Furthermore, P2X7R inhibitors can reduce ischemia-reperfusion damage in animal models[22], improve myocardial ischemia injury[22, 23], and reduce cardiac fibrosis induced by TGFβ through regulating the NLRP3/IL-1beta pathway[24]. Additionally, our previous report demonstrates that it’s important for P2X7R to regulate cardiac fibrosis in type 1 diabetes[25]. However, the potential role of P2X7R on type 2 diabetes remains unclear.

The benefits of exercise training have received widespread attention. For instance, exercise has been reported to elicit a large influx of immune cells in tumors and reduced tumor incidence and growth by more than 60% in multiple mouse models[26]. Regular exercise reduces hypersensitivity in rodent models of chronic pain[27], prevents and treats stage 1-2 hypertension in postmenopausal women[28], and manages the common effects of Multiple Sclerosis, Stroke, and Parkinson Disease[29]. In cardiovascular terms, exercise is regarded as a diagnostic and prognostic tool for chronic heart failure and an important measure for therapeutic intervention[30]. In addition, exercise is an efficacious approach for treating diabetes complications[31]. Based on previous reports, exercise can improve DCM in mice by reducing ROS production, improving mitochondrial dysfunction and maintaining energy balance[32, 33], and in diabetic rats, exercise can reduce myocardial fibrosis and improve cardiac function by inhibiting the TGF-β1/Smad signaling pathway[34], enhancing the expression of miR-486a-5p and inhibiting myocardial cell apoptosis[35]. However, whether the underlying mechanism of exercise is associated with the regulation of P2X7R levels in mice with type 2 diabetes remains elusive. Thus, this study aimed to investigate the effect of aerobic exercise on P2X7R expression and followed-by cardiac remodeling regulating in diabetic mice.

Materials And Methods

Reagents

BCL-2 (ab196495) and GAPDH (#2881S) antibodies were obtained from Abcam (Cambridge, United Kingdom), whereas Caspase-3 (#9662S) antibody was purchased from Cell Signaling Technology (Danvers, MA, United States). TGF-β (A18692) antibody was purchased from Abclonal (Wuhan, China), and Bax (ET1603-34), MMP9 (ER1706-40), MyHC (ET1702-88), and P2X7R (ER1901-99) antibodies were obtained from Huabio (Hangzhou, China). In addition, Collagen 1 (sc293182) and ANP (sc-515701) were obtained from Santa Cruz Biotechnology (Dallas, Texas, United States). Goat anti-rabbit secondary antibodies (A0208) and goat anti-mouse secondary antibodies (A0216) which were used in the Western blot and One-Step TUNEL Apoptosis Assay Kit, respectively, were purchased from Beyotime (Shanghai, China). Hematoxylin-eosin (H&E) Staining Kit and Masson's Trichrome Stain Kit were obtained from Solarbio (Beijing, China). STZ was purchased from Sigma (California, United States) and citric acid-sodium citrate buffer was purchased from Solarbio (Beijing, China).
Animals

This study was conducted out in accordance with the principles of the Guide for the Care and Use of Laboratory Animals (National Research Council, United States). The protocol was approved by the Institutional Animal Care and Use Committee, Wenzhou Medical University (wydw2016-0266). Six-week-old male lean control (db/+ ) and diabetic obese (db/db) mice with C57BL/6J background were purchased from Model Animal Research Center of Nanjing University. Purinergic P2X7 receptor knockout (P2X7R<sup>−/−</sup>) mice with C57BL/6J background were purchased from Nanjing Institute of Biological Sciences. All mice were housed in specific pathogen-free conditions. The animals were kept under a 12h/12h light-dark cycle, and they were allowed free access to food and water.

Experimental Exercise Protocol and Blood Sample Collection

After acclimatization for 1 week, P2X7R<sup>−/−</sup> mice or wild-type mice were fed either a control diet (n = 24) or a high-fat diet (HFD; HD001; Medicine, ShenZhen, China; n = 32) for 4 weeks. Next, the diabetic mice were injected with 50 mg/(kg·d) of streptomycin for five consecutive days, and they were classified as diabetic mice after observation of fasting blood glucose ≥11.1 mmol/L for two consecutive days after 1 week. Then, HFD was continued. After 12 weeks, diabetic mice were randomly divided into two groups: sedentary mice without exercise training (DM, n = 6-8) and mice with regular aerobic exercise training for 12 weeks (DM+EX, n = 8). Notably, sedentary mice were fed a standard diet, and they served as the control group (WT, n = 8). The sedentary diabetic obese (db/db) mice were fed a standard diet, and they were divided into two groups: sedentary mice (db/db, n=10) and regular aerobic exercise trained mice (db/db+EX, n=10). The exercise experiment was conducted using a small animal treadmill (#1050 RM-E57) from Columbus instruments with zero inclination. Mice in the exercise groups were trained on a motor treadmill at 5 m/min for 60 min on the first day. Initial adaptation was performed at 7 m/min for 5 days. The running speed was then increased by 1 m/min each day until the speed reached 10 m/min at the end of the training protocol[36]. Afterward, the mice were monitored to cover a daily distance at 10 m/min for the next 12 weeks, and they were trained for 5 days/week. Notably, all training sessions were performed during the afternoon (2:00–5:00 p.m.). After 12 weeks of treadmill exercise, mice were starved for 12 h, and then they were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). Blood samples were collected from the inferior vena cava into EDTA tubes. The plasma was immediately separated by centrifugation at 3000 rpm for 10 min and stored at 80°C until chemical assay analysis.

Detection of cardiac function in mice

Cardiac systolic and diastolic functions were measured using two-dimensional echocardiography. After anesthesia, the hair in the precordial area was removed, and then the ultrasonic probe was used for ultrasonic detection. Acuson-sequoia 512 was used and equipped with an acuson-15L8w probe at 12–14 MHz. Images were acquired in the M-mode and short-axis, and waveforms and related data were recorded. Ejection fraction (EF) was calculated using the following equation: \( EF = \frac{[LVEDV - LVESV]}{LVEDV} \times 100\% \). Moreover, fractional shortening (FS) was calculated using the following equation: \( FS = \frac{[LVIDd - LVIDs]}{LVIDd} \times 100\% \).

Real-Time Polymerase Chain Reaction (PCR) Analysis

Total RNA was extracted from mice hearts using TRIzol Reagent following the manufacturer’s protocol (Invitrogen Life Technologies). One microgram of total RNA from each sample was used to generate cDNAs using the RevertAidTM First Strand cDNA Synthesis Kit (#K1622; Thermo) following the manufacturer’s instructions. The resultant cDNA was amplified using SYBR Green Qpcr Super Mix-UDG kit (#RR037A; Takara). In addition, the PCR
reaction was directly monitored by the CFX96 Touch TM Real-Time PCR detection system. All results were normalized against GAPDH (B661204; Sangon Biotech, Shanghai, China).

Real-time PCR was conducted using the following primers:

P2X7R: Forward primer: CCAAGGTCAAGGCATAGCAGAGG
Reverse primer: TAGGACACCAGGCAGAGACTTCAC

MyHC: Forward primer: CAAAGGCAAGGCAAAGAAAG
Reverse primer: TCACCCCTGGAGACTTTGTC

MMP9: Forward primer: GCAGAGGCATACTTGTACCG
Reverse primer: TGATGTATGTGATGTCCCACCTTG

Collagen I: Forward primer: GAGGGCGAGTGGCTGGTCTTTC
Reverse primer: GGAGACCACGGACAGAAGG

Bax: Forward primer: CCGGGAATTGGAGATGAACT
Reverse primer: CCAGCCCATGATGGTTCTGAT

Bcl-2: Forward primer: GCTACCGTCGTAGCTTCGC
Reverse primer: CCCCACCGAACTCAAAGAAGG

Caspase-3: Forward primer: CTGACTGGAAAGCCGAAACTC
Reverse primer: CGACCCGTCCTTTGAATTTCT

ANP: Forward primer: AAGAACCTGCTAGACCACCTGGA
Reverse primer: TGCTTCCTCAGTCTGCTCAG

TGF-β: Forward primer: ACCGAAACCGCCATCTATGGAG
Reverse primer: AGGCGCATGATGTCTTGG

GAPDH: Forward primer: ACCCAGAAGACTGTGGATGG
Reverse primer: TTCAGCTCAGGGATGACCTT

Western Blot Analysis

Heart tissue samples (50-100 mg) and cardiomyocyte samples were ground and centrifuged at 12,000g for 15 min, and then the supernatants were collected. The protein samples were separated by SDS-PAGE gel and transferred to a PVDF membrane (MERCK, Germany). Membranes were blocked with a 5% fat-free milk solution for 1 h at room temperature and subsequently incubated overnight with the primary antibodies at 4°C. After washing three times,
immunoreactive bands were incubated with horseradish peroxidase-conjugated secondary antibody at room temperature for 1 h. Finally, proteins were detected via enhanced chemiluminescence (Bio-Rad, United States).

**TUNEL Staining**

The terminal deoxynucleotidyl transferase-mediated DUTP nick end labeling (TUNEL) assay was performed following the manufacturer's instructions of One-Step TUNEL Apoptosis Assay Kit (Beyotime, Shanghai, China). TUNEL positive cells were imaged under a fluorescence microscope (400× amplification; Nikon, Japan).

**Hematoxylin and Eosin Staining, Scanning Electron Microscopy, Sirius Red Staining, and Immunohistochemistry Examination**

Fresh tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Then, the tissues were cut into 5 µm sections, followed by deparaffinization and rehydration as previously described[37]. After rehydration, the sections were stained with H&E. Next, paraffin sections were stained using a Sirius Red Kit to evaluate the level of collagen deposition and fibrosis. The stained sections were then viewed under the Nikon microscope (Nikon, Japan). For immunohistochemical staining, tissue sections were deparaffinized with xylene, rehydrated in graded alcohol series, and subjected to antigen retrieval in 0.01 M of citrate buffer (pH 6.0) by microwaving. Next, the sections were placed in 3% hydrogen peroxide methanol for 30 min at room temperature. Slides were then blocked with 1% bovine serum albumin in phosphate-buffered saline for 30 min and incubated with primary antibody at 4°C overnight (P2X7R, 1:1000). Afterward, slides were incubated with peroxidase-conjugated secondary antibodies (Santa Cruz, Dallas, Texas, USA, 1:1000 dilution) for 1 h at room temperature. Finally, slides were counterstained with hematoxylin for 5 min, dehydrated, and mounted. Each image of the sections was captured using a light microscope (400× amplification; Nikon, Shinagawa, Tokyo, Japan). Notably, samples were collected on the basis of the following key points: small, fast, cold, and accurate. After rinsing, 2.5% glutaraldehyde was fixed for 2 days. After fully rinsing, 1% osmium acid was fixed for 1.5 h, and then uranium acetate block was dyed, dehydrated and soaked. Finally, the samples were sectioned using an ultrathin slicer (RMC-PXL), and then the sections were embedded. Images were viewed and acquired by using a transmission electron microscope (H-7500).

**Statistical Analysis**

All statistical analyses were performed using GraphPad Prism 8.0 software (GraphPad, San Diego, CA, United States), and all values were presented as the mean ± standard error of the mean. The normality of data was performed using Shapiro-Wilk test. In addition, the equal variance was tested using Levene's test. One-way analysis of variance followed by a multiple comparison test with Tukey's correction was used to analyze the differences within the groups. A p-value <0.05 was considered significant.

**Results**

**Aerobic exercise reduced the expression of P2X7R in cardiac tissues of DM mice**

As shown in Figure 1, P2X7R expression was significantly increased in the heart of db/db mice or HFD+STZ-induced T2DM mice compared with control mice (Figs. 1A-D) and decreased by 12-week aerobic exercise training. Consequently, we observed the same tendency of P2X7R level from the protein (Figs. 1E-H) and gene levels (Figs. 1I-J) in the DM group or DM+EX group, which was consistent with immunohistochemical staining. Collectively, these
results showed that the P2X7 receptor was upregulated in the cardiac tissues of type 2 diabetes model and downregulated by aerobic exercise.

**Changes in body weight, lipid and blood glucose levels of mice**

Various physiological parameters were measured to determine the impact of the moderate exercise regimen (Tables 1 and 2 and Fig. 2). We regularly monitored the weight of mice and found that the weight of db/db mice greatly increased compared with WT and reduced by exercise (Figs. 2A-C). In addition, the levels of total cholesterol (CHOL) and triglycerides (TRIG) were upregulated in the db/db group and downregulated in the exercise group ($p<0.05$, Table 2), whereas no significant changes in blood glucose levels were observed between the db/db and exercise groups (Fig. 2D). Moreover, similar glucose level results can be observed in the model of HFD+STZ-induced mice (Table 2 and Fig. 3E).

### Table 1

|                | CHOL (mmol/L) | TRIG (mmol/L) | CK (U/L) |
|----------------|---------------|---------------|----------|
| WT             | 1.69±0.33     | 0.88±0.26     | 732.7±181.7 |
| EX             | 1.32±0.38     | 0.93±0.33     | 754.3±297.2 |
| db/db          | 4.02±0.52*    | 2.00±0.48*    | 641.5±151.6 |
| db/db+EX       | 3.16±0.42#    | 1.15±0.25#    | 950.5±346.3 |

Data were presented as mean ± SD; *: significant difference vs. WT; #: significant difference vs. db/db; TRIG: triglyceride; CHOL: cholesterol; CK: creatine kinase. *$p<0.05$ versus the WT group, #: $p<0.05$ versus the db/db group.

### Table 2

|                | CHOL (mmol/L) | TRIG (mmol/L) | CK (U/L) |
|----------------|---------------|---------------|----------|
| WT             | 2.22±0.45     | 1.35±0.22     | 860±294.7 |
| EX             | 2.15±0.20     | 1.13±0.24     | 1037±593.2 |
| DM             | 10.57±0.67*   | 3.05±0.62*    | 805±252.2 |
| DM+EX          | 6.07±0.0.61#  | 1.74±0.13#    | 1037±349.2 |

Biochemical indicators of each experimental mouse. *$p<0.05$ versus the WT group, #: $p<0.05$ versus the DM group.

**Aerobic exercise relieved cardiac dysfunction induced by T2DM mice**
Noninvasive transthoracic echocardiography was performed to examine the cardiac function of mice 2 h before sacrifice. The data from echocardiography showed that the heart rate was not affected. As shown in Fig. 3B and Table 3, not only diastolic function disorder (as observed in IVSd and LVIDd) but also reduction of contraction function (EF%, FS%) was induced by diabetes. Consequently, these dysfunctions were substantially attenuated by aerobic exercise training in db/db mice, but no significant changes were observed between the WT group and EX group. Moreover, the consistent data trend was observed from the model of HFD+STZ-induced mice (Table 4 and Fig. 3D, F, and H). Figure 3D shows a representative echocardiogram of each group, which could be intuitively presented. Collectively, these results indicated that regular moderate intensity exercise can effectively improve cardiac function of diabetes mellitus.

| **Table 3** | Cardiac function indexes of mice in each group (±SD, n=10). |
|-------------|----------------------------------------------------------|
|             | WT            | EX            | db/db         | db/db+EX       |
| HW/BW, mg/g | 5.68±0.51     | 5.96±0.31     | 2.61±0.16*    | 3.13±0.23#     |
| IVSd, mm    | 0.56±0.06     | 0.58±0.06     | 0.67±0.08*    | 0.68±0.06      |
| LVIDd, mm   | 3.88±0.26     | 3.88±0.26     | 4.12±0.22     | 4.12±0.22      |
| LVPWd, mm   | 0.58±0.05     | 0.58±0.05     | 0.70±0.07*    | 0.63±0.02#     |
| LVIDs, mm   | 2.35±0.21     | 2.38±0.24     | 2.64±0.18*    | 2.37±0.12#     |
| EF%         | 77.06±3.90    | 76.79±3.59    | 71.20±3.28*   | 78.53±2.46#    |
| FS%         | 41.59±2.27    | 39.84±3.19    | 35.26±2.57*   | 41.43±2.17#    |

Echocardiographic assessment of cardiac function parameters of each group of experimental mice. HW/BW: heart weight/body weight; BW: weight; HW: heart weight; IVSd: diastolic interventricular septal thickness; LVIDd: diastolic left ventricle internal volume; LVPWd: left ventricular posterior wall thickness; LVIDs: left ventricular end-systolic diameter; EF%: ejection fraction; FS%: fraction shortening (n=7; *p < 0.05 versus the WT group, #p < 0.05 versus the db/db group)

| **Table 4** | Cardiac function indexes of mice in each group (±SD, n=5). |
|-------------|----------------------------------------------------------|
|             | WT            | EX            | DM            | DM+EX          |
| HW/BW, mg/g | 4.66±0.40     | 4.63±0.42     | 5.10±0.42     | 5.04±0.49      |
| IVSd, mm    | 0.73±0.05     | 0.71±0.04     | 0.69±0.04     | 0.70±0.03      |
| LVIDd, mm   | 3.99±0.13     | 3.90±0.18     | 4.29±0.22*    | 4.12±0.21      |
| LVPWd, mm   | 0.73±0.03     | 0.70±0.06     | 0.69±0.05     | 0.70±0.04      |
| LVIDs, mm   | 2.72±0.36     | 2.47±0.17     | 2.70±0.38     | 2.68±0.20      |
| EF%         | 75.1±4.07     | 73.1±4.53     | 58.83±1.17*   | 69.5±2.93#     |
| FS%         | 36.57±2.44    | 38.21±2.73    | 29±2.83*      | 33.54±2.03#    |

Echocardiographic assessment of cardiac function parameters. *p < 0.05 versus the WT group, #p < 0.05 versus the DM group.
Regular aerobic exercise inhibited myocardial apoptosis in T2DM mice

Notably, apoptosis plays a pivotal role in heart injury in DCM. As shown in cardiac TUNEL staining, more positive cells of apoptosis emerged in db/db mice than in the WT group, whereas less positive cells existed in db/db+EX mice than in db/db mice (Figs. 4A and C). The expression levels of apoptosis-related proteins, such as Caspase-3 and Bax, were increased, and anti-apoptotic protein Bcl-2 was decreased in db/db mice. Consequently, regular exercise led to the expression of Caspase-3, and the ratio of Bax to Bcl-2 was reduced (Figs. 4B, D, and E). In addition, the mRNA level of Caspase-3, Bax, and Bcl-2 showed similar results (Figs. 4F and G). We also investigated this phenomenon in HFD+STZ-induced DM mice (Fig. 5) and found that the increase of cardiac apoptosis caused by diabetes was markedly inhibited by exercise. Thus, these results indicated that exercise can effectively alleviate the apoptosis of centrifuge cells in DCM.

Regular aerobic exercise ameliorated myocardial remodeling in db/db mice

Given that aerobic exercise enhances the heart function of diabetic mice, we investigated whether it could improve cardiac remodeling. Thus, H&E staining and electron microscopy were performed to detect cardiac structure morphology. We observed significant structural abnormalities in cardiac tissues, such as disorganized myofibers caused by diabetes mellitus. In the WT group, the myocardial cells were rich in myofibrils, with clearly visible M and Z lines, round or elliptic mitochondria exhibiting many cristae and orderly arrangement, and myofilaments arranged tightly and neatly. By contrast, in cardiac tissues of the db/db group, the content of myofibrils was significantly reduced. The M and Z lines of cardiomyocytes were blurred, and the muscle filaments were broken and disordered. Moreover, we observed that mitochondrial disorder appeared as swelling deformation and cristae fracture with vacuolation in cardiac tissue of db/db mice. These disorder phenomena were markedly improved in the db/db+EX group (Figs. 6A and B).

In addition, fibrosis is an important pathological variation in DCM. The connective cardiac tissue was determined by Sirius Red staining for collagen. Compared with WT mice, the hearts from the db/db group and DM group showed apparent collagen and fibrous tissue accumulation, which were reduced by regular exercise training (Fig. 6). As for the protein level, the profibrotic makers, including TGF-β, COL-I and MMP9, and cardiac hypertrophic markers, including MyHC and ANP, were significantly elevated in the heart tissues of the db/db group or DM group. Moreover, the results of PCR for these genes level showed the same tendency. These molecular biological changes were remarkably inhibited by aerobic exercise training (Fig. 7 and Fig. 8). Overall, these experimental results suggested that aerobic exercise significantly improved myocardial remodeling in diabetic mice.

Exercise training and P2X7R deficiency ameliorated cardiac remodeling in HFD+STZ-induced T2DM mice

Considering that P2X7R expression was upregulated in the db/db group or DM group and significantly decreased after exercise, we hypothesized that P2X7R may be involved in the pathophysiological process of exercise in improving DCM in mice (Fig. 1). To further investigate the role of P2X7R in the exercise model of diabetic mice, P2X7R knockout mice were conducted and treated with HFD and STZ injection to induce the T2DM model. No significant difference was found in body weight and blood glucose level between the P2X7R−/+ +DM group and
C57BL/6 background DM mice, as well as between the P2X7R<sup>−/−</sup> +EX group and EX group (Table 5), indicating that P2X7R knockout had no effect on blood glucose level.
Table 5
Effects of exercise on the biochemistry of diabetic mice (±SD, n=6).

|                | DM     | DM+EX  | P2X7R−/− +DM | P2X7R−/− +DM+EX | P2X7R−/− +EX |
|----------------|--------|--------|--------------|-----------------|-------------|
| HW/BW, mg/g    | 5.10±0.43 | 5.04±0.49 | 5.05±0.32    | 4.73±0.38      | 5.41±0.11   |
| Glucose, mmol/L | 26.46±4.8 | 29.11±3.99 | 25.7±8.66    | 25.06±7.35     | 10.57±1.35  |
| IVSd, mm       | 0.69±0.04 | 0.70±0.03 | 0.63±0.05*   | 0.62±0.09#     | 0.75±0.03   |
| LVIDd, mm      | 4.3±0.22  | 4.117±0.209 | 4.058±0.11   | 3.927±0.20     | 4.096±0.17  |
| LVPWd, mm      | 0.69±0.049 | 0.6989±0.04122 | 0.61±0.06*   | 0.6307±0.09#   | 0.7236±0.2111 |
| LVIDs, mm      | 2.79±0.38  | 2.678±0.201 | 2.561±0.43   | 2.65±0.89      | 2.694±0.2468 |
| EF%            | 58.83±1.17 | 69.5±2.929* | 67.71±3.77*  | 75.13±3.23#&   | 76.83±3.19  |
| FS%            | 29±2.83   | 33.54±2.03* | 33.8±2.17*   | 38.4±2.41#     | 39.75±2.63  |

Echocardiographic assessment of cardiac function parameters of each group of experimental mice. BW: body weight; HW: heart weight; HW/BW: heart weight/body weight; IVSd: diastolic interventricular septal thickness; LVIDd: diastolic left ventricle internal volume; LVPWd: left ventricular posterior wall thickness; LVIDs: left ventricular end-systolic diameter; EF%: ejection fraction; FS%: fraction shortening (n=7; *p < 0.05 versus the DM group, #p < 0.05 versus the DM+EX group)

In addition, all mice were tested for echocardiography 2 h before sacrifice to evaluate cardiac function. As shown in Table 5, under basal conditions, the P2X7R−/− group had no effect on cardiac function compared with the WT group (Table 5). Moreover, systolic dysfunction (as shown in EF% and FS% indices) was observed in the DM group, which improved by P2X7R deficiency.

With regard to changes in myocardial structure, apparent structural abnormalities, such as disorderly arranged muscle fiber, were observed in the HFD+STZ-induced DM group and typically reversed by P2X7R knock out or aerobic exercise treatment (Figs. 9A and B). In addition, the hearts of diabetic mice exhibited evident deposition of fibrous tissue and collagen tissue. Similarly, the expression of profibrotic indicators (TGF-β, COL-1, and MMP9) and cardiac hypertrophic markers (MyHC and ANP) were evidently increased in diabetic mice. Interestingly, these pathophysiological changes were reversed by the DM+EX group or P2X7R−/−+DM group (Fig. 9 and Fig. 10). These results suggested that exercise or the deficiency of P2X7R can effectively improve myocardial fibrosis and hypertrophy. Next, we further assessed the levels of apoptosis in cardiac tissues. Based on the data obtained from TUNEL staining, P2X7R knockout or exercise training had positive effects on reducing myocardial apoptosis and protecting the survival of cardiomyocytes in diabetic mice (Fig. 11A and B). Moreover, HFD+STZ treatment increased Caspase 3 and Bax expression and inhibited the expression of Bcl-2 in myocardial tissue. And as we expected, these abnormal changes were decreased in the P2X7R−/− +DM group or DM+EX group (Fig. 11C-G).

Considering that aerobic exercise typically reduced P2X7R expression and ameliorated cardiac remodeling in diabetic mice, we evaluated the effect of P2X7R knockout combined with exercise treatment on T2DM mice. Strikingly, in the P2X7R−/−+DM+EX group, we observed evident improvement in cardiac dysfunction, dramatically alleviated cardiac fibrosis and hypertrophy, and decreased myocardial apoptosis level compared with the DM+EX group (Fig. 10 and Fig. 11). Compared with the P2X7R−/−+DM group, the P2X7R−/−+DM+EX group represented a lower level of cardiac fibrosis (Figs. 9A and B). The expression of TGF-β at the protein level and the level of TGF-β and ANP at the genetic level evidently decreased in the P2X7R−/−+DM+EX group, while it did not significantly differ from other indicators (Fig. 9 and 10). These results indicated that exercise can effectively alleviate myocardial apoptosis and alleviate myocardial fibrosis at least in part by inhibiting P2X7R.
Discussion

In this study, we revealed the role of aerobic exercise on a mouse model of T2DM with or without P2X7R deficiency. We found that P2X7R expression significantly increased in db/db mice and HFD+STZ-induced DM mice, accompanied by cardiac dysfunction and enhancement of myocardial fibrosis and apoptosis. Aerobic exercise significantly inhibited P2X7R expression, reduced myocardial apoptosis and relieved cardiac fibrosis and hypertrophy. In addition, depletion of P2X7R effectively reversed these abnormal changes. We showed that P2X7R−/− +DM+EX displayed more evident improvement in cardiac remodeling, dysfunction, and apoptosis in T2DM mice compared with the DM+EX group, suggesting the beneficial effect of exercise training on DCM could partly inhibit P2X7R. Collectively, our data revealed the pivotal role of P2X7R in the pathophysiology of T2DM, and aerobic exercise can improve myocardial remodeling, at least partly via P2X7R-dependent mechanisms in DCM.

Myocardial remodeling is an important feature of many cardiovascular diseases, which primarily appears as interstitial, perivascular fibrosis and hypertrophy and impaired heart function[38]. In DCM, apparent cardiac abnormality lies different mechanisms, namely, inflammation, mitochondrial dysfunction, and apoptosis, which ultimately lead to the extracellular cardiac remodeling and eventually heart failure[39]. Reversing these detrimental processes will improve the cardiac function of patients with diabetes mellitus. Here, we observed consistent pathological changes of cardiac tissues from a mouse model of db/db mice or HFD+STZ-induced T2DM mice. Interestingly, in both models of T2DM mice, we found that P2X7R was abundantly expressed in cardiac tissues. In addition, the depletion of P2X7R effectively alleviated cardiac remodeling and improved cardiac systolic dysfunction in T2DM mice. This result was consistent with our previous study in the model of T1DM[25], which indicates P2X7R deficiency ameliorates cardiac injury and remodeling in mice by PKCβ and ERK. Collectively, P2X7R, as a potential target for the therapeutic management, plays an important role in the regulation of heart function in DCM.

Mechanically, among the pathological mechanisms of DCM that have been reported[40], apoptosis of cardiomyocytes is the main cause of DCM progression. In this study, we confirmed that the expression of apoptosis-related proteins and the number of apoptosis-positive cells in TUNEL staining were increased in db/db mice or HFD+STZ-treated T2DM mice and bluntly decreased in the exercise group.

For decades, regular exercise has been found to delay or prevent some complications caused by diabetes, although it induces physiological cardiac hypertrophy through cardiac cell growth[41, 42]. A growing body of evidence indicates that aerobic exercise can improve DCM. In brief, regular aerobic exercise presents significant improvements in cardiac function and remodeling, improved glucose and insulin metabolism[4, 43], and decreases cardiac fibrosis, apoptosis, and oxidative stress, thereby reducing the risk factors for cardiovascular disease[42]. In addition, clinical significance of exercise for DCM is observed[44, 45], and over the long term, 150 min or more of moderate-to-vigorous intensity aerobic activity every week is recommended to be the protective strategy against the development of DCM according to the 2019 American Diabetes Association guidelines[46]. Consistent with the above-mentioned results, here we elucidated that exercise can reduce myocardial cell apoptosis and myocardial fibrosis in diabetic mice and improve cardiac dysfunction in T2DM. Thus, exercise plays an essential role in the regulation of cardiac function.

For the potential protective mechanism roles of exercise against DCM, much of literature in this field indicates that exercise can regulate cardiomyocyte metabolism by increasing GLUT-4 expression or reducing Forkhead box protein O1[47] and play a role in the regulation of calcium, mitochondrial function, oxidative stress, and cardiac ultrastructural changes[48, 49]. In the present study, we revealed that increased P2X7R expression caused by diabetes was inhibited by regular exercise, and the depletion of P2X7R effectively reversed cardiac remodeling, indicating that exercise improves cardiac dysfunction caused by diabetes by regulating P2X7R. These data provide
supportive evidence which P2X7R is another potential target involved in the effect of exercise on DCM. Notably, we next further underscored this effect in HFD+STZ-induced mice by combining P2X7R knockout with exercise treatment. More prominent beneficial effect on the heart was observed in the P2X7R−−/+DM+EX group compared with the DM+EX group. Moreover, compared with the P2X7R−−+DM group, the P2X7R−−+DM+EX group reduced myocardial collagen deposition, which indicated that exercise alleviated cardiac remodeling in DCM partly by inhibiting P2X7R expression.

**Conclusions**

This study systematically revealed the role of exercise in reducing P2X7R expression, modulating cardiac fibrosis and apoptosis, and improving cardiac dysfunction in DCM at least partly by inhibiting the P2X7R levels of cardiac tissues. Therefore, exercise training, as an effective non-pharmacologic measure made out of a tailored exercise prescription would positively affect DCM management in the future.

**Abbreviations**

DCM
diabetic cardiomyopathy
P2X7R
P2X7 purinergic receptors
HFD
high-fat diet
STZ
streptozotin
T2DM
type 2 diabetes mellitus
CAD
coronary artery disease
ROS
reactive oxygen species
WT
the control group
db/db
db/db mice
EX
excise
db/db+EX
db/db mice plus exercise
DM
HFD+STZ induced T2DM mice
DM+EX
HFD+STZ induced T2DM mice plus exercise
P2X7R−−
Purinergic P2X7 receptor knockout mice
P2X7R−−+ EX
Purinergic P2X7 receptor knockout mice plus exercise
P2X7R−/−+DM

Purinergic P2X7 receptor knockout and HFD+STZ induced T2DM mice
P2X7R−/−+DM+EX

Purinergic P2X7 receptor knockout and HFD+STZ induced T2DM mice plus exercise

HW/BW
heart weight/body weight

BW
weight

HW
heart weight

EF
ejection fraction

FS
fractional shortening

LVIDd
diastolic left ventricle internal volume

LVIDs
left ventricular end-systolic diameter

IVSd
diastolic interventricular septal thickness

LVPWd
left ventricular posterior wall thickness

PCR
Real-Time Polymerase Chain Reaction

TUNEL
terminal deoxynucleotidyl transferase-mediated DUTP nick end labeling

CHOL
total cholesterol

TRIG
triglycerides

CK
creatine kinase

TGF-β
fibrotic growth factor beta

MMP9
matrix metalloproteinase-9

COL-1
collagen I

MyHC
myosin heavy chain

Bcl-2
B cell lymphoma/leukemia-2

Bax
Bcl-2 associated X protein
Caspase-3
cysteiny1 aspartate specific proteinase-3
GAPDH
glyceraldehyde-phosphate dehydrogenase
H&E
Hematoxylin and Eosin
RNA
ribonucleic acid

Declarations

Ethics approval and consent to participate
This study was performed in accordance with the principles of the Guide for the Care and Use of Laboratory Animals (National Research Council, United States). The experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee, Wenzhou Medical University (wydw2016-0266).

Consent for publication
All authors gave their consent for publication.

Availability of data and materials
All data generated or analyzed during this study were included in this article.

Competing interests
The authors declare that they have no competing interests.

Funding
This study was supported by the National Natural Science Foundation of China (grant nos. 82070446 and 81670227), the Wenzhou Science and Technology Bureau (grant nos. Y2020242), and the Key Research and Development Program of Zhejiang (grant no. 2019C03012).

Authors' contributions
WT designed the study, performed experiments. WT and LJM analyzed data and drafted the manuscript. WT, LJM, LH, ZX, WLY, ZSY and LXS read and revised the manuscript. HZQ and WYH conducted a critical revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgements
Not applicable.

Author details
References

1. Ritchie RH, Abel ED. Basic Mechanisms of Diabetic Heart Disease. Circ Res. 2020;126(11):1501–25.
2. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271–81.
3. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract. 2014;103(2):137–49.
4. Zheng J, Cheng J, Zheng S, Zhang L, Guo X, Zhang J, Xiao X: Physical Exercise and Its Protective Effects on Diabetic Cardiomyopathy: What Is the Evidence? Front Endocrinol (Lausanne) 2018, 9:729.
5. Zhang X, Hao Y. Beneficial Effects of Echinacoside on Diabetic Cardiomyopathy in Diabetic Db/Db Mice. Drug Des Devel Ther. 2020;14:5575–87.
6. Liu F, Song R, Feng Y, Guo J, Chen Y, Zhang Y, Chen T, Wang Y, Huang Y, Li C-Y, et al. Uregulation of MG53 induces diabetic cardiomyopathy through transcriptional activation of peroxisome proliferation-activated receptor α. Circulation. 2015;131(9):795–804.
7. Bugger H, Abel ED. Molecular mechanisms of diabetic cardiomyopathy. Diabetologia. 2014;57(4):660–71.
8. Gibb AA, Hill BG. Metabolic Coordination of Physiological and Pathological Cardiac Remodeling. Circulation research. 2018;123(1):107–28.
9. Mellor KM, Bell JR, Young MJ, Ritchie RH, Delbridge LMD. Myocardial autophagy activation and suppressed survival signaling is associated with insulin resistance in fructose-fed mice. J Mol Cell Cardiol. 2011;50(6):1035–43.
10. Chowdhry MF, Vohra HA, Galiñanes M. Diabetes increases apoptosis and necrosis in both ischemic and nonischemic human myocardium: role of caspases and poly-adenosine diphosphate-ribose polymerase. J Thorac Cardiovasc Surg 2007, 134(1).
11. Miras-Portugal MT, Sebastian-Serrano A, de Diego Garcia L, Diaz-Hernandez M. Neuronal P2X7 Receptor: Involvement in Neuronal Physiology and Pathology. J Neurosci. 2017;37(30):7063–72.
12. Garre JM, Silva HM, Lafaille JJ, Yang G. P2X7 receptor inhibition ameliorates dendritic spine pathology and social behavioral deficits in Rett syndrome mice. Nat Commun. 2020;11(1):1784.
13. Solini A, Novak I. Role of the P2X7 receptor in the pathogenesis of type 2 diabetes and its microvascular complications. Curr Opin Pharmacol. 2019;47:75–81.
14. Wu Y, Zhang Y, Zhang J, Zhai T, Hu J, Luo H, Zhou H, Zhang Q, Zhou Z, Liu F. Cathelicidin aggravates myocardial ischemia/reperfusion injury via activating TLR4 signaling and P2X7R/NLRP3 inflammasome. J Mol Cell Cardiol. 2020;139:75–86.
15. Baeza-Raja B, Goodyear A, Liu X, Lam K, Yamamoto L, Li Y, Dodson GS, Takeuchi T, Kisseleva T, Brenner DA, et al. Pharmacological inhibition of P2RX7 ameliorates liver injury by reducing inflammation and fibrosis. PloS one. 2020;15(6):e0234038.

16. Huang C, Yu W, Cui H, Wang Y, Zhang L, Han F, Huang T. P2X7 blockade attenuates mouse liver fibrosis. Mol Med Rep. 2014;9(1):57–62.

17. Goncalves RG, Gabrich L, Rosario A Jr; Takiya CM, Ferreira ML, Chiarini LB, Persechini PM, Coutinho-Silva R, Leite M Jr. The role of purinergic P2X7 receptors in the inflammation and fibrosis of unilateral ureteral obstruction in mice. Kidney Int. 2006;70(9):1599–606.

18. Chen Z, He L, Li L, Chen L. The P2X7 purinergic receptor: An emerging therapeutic target in cardiovascular diseases. Clin Chim Acta. 2018;479:196–207.

19. Ding L, Gong C, Zhao J, Liu X, Li T, Rao S, Wang S, Liu Y, Peng S, Xiao W, et al. Noncoding transcribed ultraconserved region (T-UCR) U2.48+ is a novel regulator of high-fat diet induced myocardial ischemia/reperfusion injury. Journal of cellular physiology. 2019;234(6):9849–61.

20. Peng K, Liu L, Wei D, Lv Y, Wang G, Xiong W, Wang X, Altaf A, Wang L, He D, et al. P2X7R is involved in the progression of atherosclerosis by promoting NLRP3 inflammasome activation. Int J Mol Med. 2015;35(5):1179–88.

21. Zhou J, Zhou Z, Liu X, Yin H-Y, Tang Y, Cao X. P2X7 Receptor-Mediated Inflammation in Cardiovascular Disease. Front Pharmacol. 2021;12:654425.

22. Granado M, Amor S, Montoya JJ, Monge L, Fernandez N, Garcia-Villalon AL. Altered expression of P2Y2 and P2X7 purinergic receptors in the isolated rat heart mediates ischemia-reperfusion injury. Vascul Pharmacol. 2015;73:96–103.

23. Tu G, Li G, Peng H, Hu J, Liu J, Kong F, Liu S, Gao Y, Xu C, Xu X, et al. P2X(7) inhibition in stellate ganglia prevents the increased sympathoexcitatory reflex via sensory-sympathetic coupling induced by myocardial ischemic injury. Brain Res Bull. 2013;96:71–85.

24. Zhou J, Tian G, Quan Y, Li J, Wang X, Wu W, Li M, Liu X. Inhibition of P2X7 Purinergic Receptor Ameliorates Cardiac Fibrosis by Suppressing NLRP3/IL-1 Pathway. Oxidative Med Cell Longev. 2020;2020:7956274.

25. Huang S, Wang W, Li L, Wang T, Zhao Y, Lin Y, Huang W, Wang Y, Huang Z. P2X7 Receptor Deficiency Ameliorates STZ-induced Cardiac Damage and Remodeling Through PKCbeta and ERK. Front Cell Dev Biol. 2021;9:692028.

26. Idorn M, Thor Straten P. Exercise and cancer: from "healthy" to "therapeutic". Cancer Immunol Immunother. 2017;66(5):667–71.

27. Pitcher MH. The Impact of Exercise in Rodent Models of Chronic Pain. Curr Osteoporos Rep. 2018;16(4):344–59.

28. Lin Y-Y, Lee S-D. Cardiovascular Benefits of Exercise Training in Postmenopausal Hypertension. International journal of molecular sciences 2018, 19(9).

29. Kim Y, Lai B, Mehta T, Thirumalai M, Padalabalanarayanan S, Rimmer JH, Motl RW. Exercise Training Guidelines for Multiple Sclerosis, Stroke, and Parkinson Disease: Rapid Review and Synthesis. Am J Phys Med Rehabil. 2019;98(7):613–21.

30. Cattadori G, Segurini C, Picozzi A, Padeletti L, Anzà C. Exercise and heart failure: an update. ESC Heart Fail. 2018;5(2):222–32.

31. Yaribeygi H, Butler AE, Sahebkar A. Aerobic exercise can modulate the underlying mechanisms involved in the development of diabetic complications. Journal of cellular physiology. 2019;234(8):12508–15.
32. Mahmoud AM. Exercise Ameliorates Metabolic Disturbances and Oxidative Stress in Diabetic Cardiomyopathy: Possible Underlying Mechanisms. Adv Exp Med Biol. 2017;999:207–30.

33. Wang SY, Zhu S, Wu J, Zhang M, Xu Y, Xu W, Cui J, Yu B, Cao W, Liu J. Exercise enhances cardiac function by improving mitochondrial dysfunction and maintaining energy homoeostasis in the development of diabetic cardiomyopathy. J Mol Med (Berl). 2020;98(2):245–61.

34. Wang SQ, Li D, Yuan Y. Long-term moderate intensity exercise alleviates myocardial fibrosis in type 2 diabetic rats via inhibitions of oxidative stress and TGF-beta1/Smad pathway. J Physiol Sci. 2019;69(6):861–73.

35. Sun D, Wang H, Su Y, Lin J, Zhang M, Man W, Song X, Zhang L, Guo B, Hao K, et al. Exercise alleviates cardiac remodelling in diabetic cardiomyopathy via the mir-486a-5p-Mst1 pathway. Iran J Basic Med Sci. 2021;24(2):150–9.

36. Yurinskaya VE, Rubashkin AA, Vereninov AA. Balance of unidirectional monovalent ion fluxes in cells undergoing apoptosis: why does Na+/K+ pump suppression not cause cell swelling? J Physiol. 2011;589(Pt 9):2197–211.

37. You S, Qian J, Sun C, Zhang H, Ye S, Chen T, Xu Z, Wang J, Huang W, Liang G. An Aza resveratrol-chalcone derivative 6b protects mice against diabetic cardiomyopathy by alleviating inflammation and oxidative stress. J Cell Mol Med. 2018;22(3):1931–43.

38. Zhou J, Tian G, Quan Y, Li J, Wang X, Wu W, Li M, Liu X. Inhibition of P2X7 Purinergic Receptor Ameliorates Cardiac Fibrosis by Suppressing NLRP3/IL-1beta Pathway. Oxid Med Cell Longev. 2020;2020:7956274.

39. Palomer X, Pizarro-Delgado J, Vazquez-Carrera M. Emerging Actors in Diabetic Cardiomyopathy: Heartbreaker Biomarkers or Therapeutic Targets? Trends Pharmacol Sci. 2018;39(5):452–67.

40. Parim B, Sathibabu Uddandrao VV, Saravanan G. Diabetic cardiomyopathy: molecular mechanisms, detrimental effects of conventional treatment, and beneficial effects of natural therapy. Heart Fail Rev. 2019;24(2):279–99.

41. McGavock JM, Eves ND, Mandic S, Glenn NM, Quinney HA, Haykowsky MJ. The role of exercise in the treatment of cardiovascular disease associated with type 2 diabetes mellitus. Sports medicine (Auckland NZ). 2004;34(1):27–48.

42. Seo DY, Ko JR, Jang JE, Kim TN, Youm JB, Kwak H-B, Bae JH, Kim AH, Ko KS, Rhee BD, et al: Exercise as A Potential Therapeutic Target for Diabetic Cardiomyopathy: Insight into the Underlying Mechanisms. International journal of molecular sciences 2019, 20(24).

43. Verboven M, Van Ryckeghem L, Belkhouribchia J, Dendale P, Eijnde BO, Hansen D, Bito V. Effect of Exercise Intervention on Cardiac Function in Type 2 Diabetes Mellitus: A Systematic Review. Sports Med. 2019;49(2):255–68.

44. Taylor JD, Fletcher JP, Mathis RA, Cade WT. Effects of moderate- versus high-intensity exercise training on physical fitness and physical function in people with type 2 diabetes: a randomized clinical trial. Phys Ther. 2014;94(12):1720–30.

45. da Silva DE, Grande AJ, Roever L, Tse G, Liu T, Biondi-Zoccai G, de Farias JM. High-Intensity Interval Training in Patients with Type 2 Diabetes Mellitus: a Systematic Review. Curr Atheroscler Rep. 2019;21(2):8.

46. 3. Prevention or Delay of Type 2 Diabetes. Diabetes Care. 2019;42(Suppl 1):29–33.

47. Röhling M, Herder C, Stemper T, Müßig K. Influence of Acute and Chronic Exercise on Glucose Uptake. J Diabetes Res. 2016;2016:2868652.

48. Hafstad AD, Boardman N, Aasum E. How exercise may amend metabolic disturbances in diabetic cardiomyopathy. Antioxid Redox Signal. 2015;22(17):1587–605.
Regular aerobic exercise reduced the P2X7R levels in db/db mice or HFD+STZ-induced T2DM mice. (A, B) Immunohistochemistry of P2X7R. (C, D) Immunohistochemical analysis of P2X7R. (E, F) Representative Western blot analysis of P2X7R. (G-J) semi-quantification of protein and mRNA level of P2X7R. *p < 0.05 versus the WT group, #p < 0.05 versus the db/db group or DM group. WT: the control group.
Figure 2

Changes in general characteristics of mice. (A) The appearance of mice after exercise training. (B) Weight of mice after exercise training. (C–F) Body weight and blood glucose level during exercise training in mice. *p < 0.05 versus the WT group, #p < 0.05 versus the db/db group.

Figure 3

Exercise improved heart function in db/db mice or DM mice. (A, C) Cardiac morphology after exercise in db/db or DM mice. (B, D) M-mode echocardiography in mice. (E–H) Cardiac function in mice. *p < 0.05 versus the WT group, #p < 0.05 versus the DM group. EF%: ejection fraction; FS%: fraction shortening.
Figure 4

Exercise alleviated cardiomyocyte apoptosis in db/db mice. (A) TUNEL staining and quantitative analysis of myocardial tissue in mice (C); (B, D, E) Western blot analysis was performed to detect apoptosis-related proteins (Caspase-3, Bcl-2, and Bax). (F, G) Gene expression of apoptotic protein. *p < 0.05 versus the WT group, #p < 0.05 versus the db/db group.
Figure 5

Exercise alleviated cardiomyocyte apoptosis in HFD+STZ-induced T2DM mice. (A, C) TUNEL staining and quantitative analysis of myocardial tissue in mice; (B, D, E) Western blot analysis was performed to detect apoptosis-related proteins (Caspase-3 and Bax) and anti-apoptotic protein (Bcl-2); (F, G) Gene expression of apoptosis-related proteins. *p < 0.05 versus the WT group, #p < 0.05 versus the DM group.
Figure 6

Exercise mitigated myocardial remodeling in diabetic mice. (A, B) TEM images of mouse myocardial tissue, hematoxylin–eosin (H&E) and Sirius red quantitative statistical images of Sirius red detecting myocardial fibrosis (C, D). *p < 0.05 versus the WT group, #p < 0.05 versus the db/db group.
Figure 7

Exercise weakened myocardial remodeling in diabetic mice. (A–F) Protein expression of fibrotic markers and hypertrophy in myocardial tissue and Statistical Graph. (G–J) Gene expression of myocardial hypertrophic markers and fibrosis in myocardial tissue. *p < 0.05 versus the WT group, #p < 0.05 versus the db/db group.
Figure 8

Aerobic exercise ameliorated myocardial remodeling in DM mice. (A–J) Changes in protein and mRNA levels of hypertrophic fibrosis indicators. *p < 0.05 versus the WT group, #p < 0.05 versus the DM group.
Myocardial remodeling and collagen deposition in the T2DM model of P2X7R knockout mice induced by HFD and STZ injection. (A, B) Hematoxylin–eosin (H&E) and Sirius red staining quantitative statistical images of Sirius red detecting myocardial fibrosis. (C–G) Proteins levels of fibrotic markers (e.g., collagen I, TGF-β, and MMP9) and hypertrophic markers (e.g., MyHC) in myocardial tissues were measured using Western blotting. *p < 0.05 versus the DM group, #p < 0.05 versus the DM+EX group.
Figure 10

Real-time polymerase chain reaction (PCR) showed levels of MyHC, ANP, TGF-β, MMP-9, and COL-1 (A-E). *p<0.05 versus the DM group, #p<0.05 versus the DM+EX group. &p<0.05 versus the P2X7R−/−+DM group.
Figure 11

(A, B) The images and quantitative statistics of TUNEL staining for apoptosis of myocardial cells in diabetic mice. (C–F) Statistical map of protein and gene expression of apoptosis-related protein (Caspase-3, Bax, and Bcl-2) in myocardial tissues were measured using Western blotting and real-time polymerase chain reaction (PCR). *p<0.05 versus the DM group, #p<0.05 versus the DM+EX group.