Association of AT1R□Mediated TGF□β1/Smad Axis Down□regulation with Anti□fibrotic and Functional Benefits of Extended Exercise After Myocardial Infarction

Keywords
Exercise training, Myocardial infarction, Cardiac fibrosis, TGF-β1, Smad signaling, Angiotensin II type 1 receptor, Rat model, Cardiac pathology, Echocardiography, Systolic function

Abstract

Introduction
Exercise training has cardioprotective effects after myocardial infarction (MI), but the associated molecular pathways remain incompletely defined. The novel aspect of this work is the comparison of short-term and extended post-MI ET while examining the angiotensin II type 1 receptor (AT1R)/TGF-β1/Smad axis as an associated anti-fibrotic pathway.

Material and methods
Sprague-Dawley rats were assigned to four groups: sham control, MI-sedentary, MI with 4-week Exercise training (MIex1), and MI with 12-week of exercise training (MIex3). ET began three days after coronary artery ligation. After 12 weeks, echocardiography and hemodynamic measurements were performed, and left ventricular (LV) tissue was collected for histological and molecular analyses.

Results
Compared with MI-sedentary rats, MIex1 rats showed increased LV systolic pressure, ±dP/dtmax, ejection fraction, and fractional shortening, along with decreased LV end-diastolic pressure and LV diameters. Twelve-week Exercise training further enhanced functional outcomes. Both ET groups reduced collagen volume fraction and collagen I/III expression. ET was also associated with lower cardiac Ang II, AT1R, TGF-β1, and phosphorylated Smad2/3, with stronger effects after 12 weeks of Exercise training.

Conclusions
Exercise reduces myocardial fibrosis following MI and preserves cardiac function, an effect that may be amplified by extended exercise training. Downregulation of AT1R-mediated TGF-β1/Smad signaling may be associated with these cardioprotective effects.

Association of AT1R-Mediated TGF-β1/Smad Axis Down-regulation with Anti-fibrotic and Functional Benefits of Extended Exercise After Myocardial Infarction

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ABSTRACT

Introduction: Exercise training (ET) has cardioprotective effects after myocardial infarction (MI), but the associated molecular pathways remain incompletely defined. The novel aspect of this work is the comparison of short-term and extended post-MI ET while examining the angiotensin II type 1 receptor (AT1R)/TGF-β1/Smad axis as an associated anti-fibrotic pathway. **Methods:** Sprague-Dawley rats were assigned to four groups: sham control, MI-sedentary, MI with 4-week ET (MIex1), and MI with 12-week ET (MIex3). ET began three days after coronary artery ligation. After 12 weeks, echocardiography and hemodynamic measurements were performed, and left ventricular (LV) tissue was collected for histological and molecular analyses. **Results:** Compared with MI-sedentary rats, MIex1 rats showed increased LV systolic pressure, ±dP/dtmax, ejection fraction, and fractional shortening, along with decreased LV end-diastolic pressure and LV diameters. Twelve-week ET further enhanced functional outcomes. Both ET groups reduced collagen volume fraction and collagen I/III expression. ET was also associated with lower cardiac Ang II, AT1R, TGF-β1, and phosphorylated Smad2/3, with stronger effects after 12 weeks of ET**. Conclusions:** Exercise reduces myocardial fibrosis following MI and preserves cardiac function, an effect that may be amplified by extended exercise training. Downregulation of AT1R-mediated TGF-β1/Smad signaling may be associated with these cardioprotective effects.

Keywords: Exercise training; Myocardial infarction; Cardiac fibrosis; TGF-β1; Smad signaling; Angiotensin II type 1 receptor; Rat model; Cardiac pathology;

Echocardiography; Systolic function

Highlights:

- Extended exercise (12 weeks) provides greater anti-fibrotic benefits than short-term exercise (4 weeks) after MI
- Exercise improves post-MI LV structure and systolic function
- Exercise attenuates collagen deposition and collagen I/III expression
- Anti-fibrotic effects coincide with lower Ang II/AT1R/TGF-β1/Smad signaling

1. Introduction

Despite modern reperfusion and guideline-directed drugs, post-MI LV remodeling still drives a substantial burden of heart failure [1]. Initially adaptive, the process soon becomes maladaptive, triggering fibrosis, hypertrophy, scar thinning, chamber dilation, and progressive dysfunction, prompting an urgent search for effective anti-remodeling therapies[2, 3].

Post-MI remodeling is primarily driven by fibrosis, characterized by excessive extracellular matrix deposition and hyperactive fibroblasts that differentiate into myofibroblasts, acquiring a pro-fibrotic phenotype [3, 5]. Central to this process is the renin-angiotensin axis; infarcted myocardium exhibits surging angiotensin II (Ang II) and upregulated AT1R, which activate the TGF- β 1/Smad cascade [5-7]. Ligand binding to the TGF-β1 receptor triggers phosphorylation of Smad2/3, which then heterodimerize with Smad4 and enter the nucleus to transcribe pro-fibrotic genes. This includes collagen, fibronectin, connective tissue growth factor (CTGF), and plasminogen activator inhibitor-1 (PAI-1). CTGF stabilizes collagen deposition, and

PAI-1 inhibits matrix degradation. Conversely, Smad7 acts as an endogenous brake, disrupting the pathway through negative feedback [7-9].

Previous work in atrial fibrillation and radiation models confirmed that Ang II, via AT1R, amplifies Smad2/3 while silencing Smad7. These changes culminate in accelerated cardiac fibrosis [10-12]. Targeting this AT1R → TGF- β 1/Smad axis, therefore, represents a rational anti-fibrotic strategy after MI.

Evidence suggests that exercise-based rehabilitation may positively influence the clinical progression of post-MI remodeling and enhance cardiac function [13-15]. ET is shown to reduce the risk of death and reinfarction as a secondary preventive treatment for MI [15], while also normalizing cardiac and circulating RAAS in cases of heart failure and MI [16,17]. The benefits of exercise may include reduced fibrosis, inflammation, and oxidative stress, and stabilized mitochondrial biogenesis [18-20]. Nonetheless, there is a paucity of data on the molecular signaling mechanisms underlying ET-induced cardioprotection in post-MI hearts.

The present study, therefore, aimed to determine: (1) whether ET can attenuate post-MI cardiac fibrosis and remodeling; (2) whether ET is associated with reduced Ang II/AT1R/TGF- β 1/Smad signaling; and (3) whether prolonged ET demonstrates greater benefits for myocardial fibrosis and cardiac function than short-term ET.

2. Materials and methods

All experimental procedures were performed in accordance with the National

Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978). All reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified in the text.

2.1 Study design and location

This animal study was conducted at the Guangdong Cardiovascular Institute Animal Laboratory (Guangzhou, China) between September 2021 and March 2022. All experimental procedures were performed in accordance with the NIH Guide and were approved by the Institutional Animal Care and Use Committee of Guangdong Provincial People's Hospital of Guangdong Academy of Medical Sciences.

2.2 Animals and MI model

Male Sprague-Dawley rats weighing 200–250g were obtained from the Experimental Animal Center of Sun Yat-sen University (Guangzhou, China). The MI rat model was induced by ligation of the left coronary artery according to a method described previously [21].

2.3 Experimental groups and exercise protocol

As illustrated in **Figure 1**, a total of 80 rats were randomly assigned into four groups: Sham-operation control (Sham, n=20), MI-Sedentary (MI, n=20), MI plus 4-week ET (MIex$_1$, n=20), and MI plus 12-week ET (MIex$_3$, n=20). The MIex$_1$ group underwent exercise training for the first 4 weeks post-MI and then remained sedentary for the subsequent 8 weeks until the 12-week endpoint. In contrast, the MIex$_3$ group underwent exercise training continuously for all 12 weeks.

According to previous studies [17, 22], ET was initiated in all MIex groups 3 days after MI and performed 5 days per week on a rodent treadmill. Treadmill speed and duration were initiated at 10 m/min, 5° incline for 10 minutes per session, then gradually increased to 16 m/min and 50 minutes per session and maintained at this level throughout the experiment.

2.4 Echocardiographic study

After 12 weeks, all rats underwent echocardiographic measurements using a system equipped with a 10-MHz transducer (SonoHeart Elite, SonoSite, Bothell, WA) as described previously [23].

2.5 Hemodynamic measurements

Following the end of 12 weeks, a terminal surgical procedure was performed to evaluate left ventricular pressure and contractile properties as described previously [24].

2.6 Radioimmunoassay of Ang II accumulation

LV tissues (50 mg) were homogenized in cold acetic acid, centrifuged, and processed according to the instructions of the 125I-CAMP radioimmunoassay kit (Medical College of Sun Yat-sen University, Guangzhou, China) to quantify Ang II accumulation.

2.7 Tissue collection

After completion of hemodynamic measurements, rats were euthanized by intravenous injection of pentobarbital sodium (150 mg/kg). The hearts were rapidly excised and rinsed with ice-cold PBS. The left ventricle (LV) was dissected from the right ventricle

and atria and then cut into three transverse sections: the apical section was fixed in 10% neutral buffered formalin for histological analysis; the mid-portion was snap-frozen in liquid nitrogen and stored at -80 C for protein extraction; and the basal section was stored in RNAlater (Thermo Fisher) at -80 C for RNA extraction.

2.8 Masson trichrome staining and collagen volume fraction

LV tissues were fixed in 10% neutral buffered formalin for 24 hours, dehydrated through a graded ethanol series (70%, 80%, 95%, 100%), cleared in xylene, and embedded in paraffin wax (melting point 56-58°C) as described previously [25]. Sections (4 μm) were cut using a rotary microtome (Leica RM2255, Germany) and mounted on glass slides. Masson trichrome staining of the paraffin sections was performed as previously described [25] using a commercial kit (Sigma-Aldrich, HT15). Briefly, sections were stained with Weigert's iron hematoxylin for 10 minutes, Biebrich scarlet acid fuchsin for 5 minutes, phosphomolybdic-phosphotungstic acid for 10 minutes, and aniline blue for 5 minutes, with washes between steps.

2.9 Quantitative real-time polymerase chain reaction (qRT-PCR)

After all experimental treatments, total RNA from rat LV tissues was isolated using TRIzol reagent (Aidlab Biotechnologies, Beijing, China) according to the manufacturer's protocol, and mRNA fold change relative to GAPDH was calculated as described previously [26]. All qRT-PCR reactions were performed in triplicate for each biological sample. Three independent biological replicates (n=3 per group) were used for qRT-PCR analysis.

PCR primers were as follows: TGF-β1: forward 5'-ACA TTG ACT TCC GCA AGG

AC-3' and reverse 5'-TAG TAC ACG ATG GGC AGT GG-3'; Smad3: forward 5'-GTG GCA TAC TGG GAG GAG AA-3' and reverse 5'-GAT GGA GAA ACC AGG GAA CA-3'; AT1R: forward 5'-GCT TGG ATC TGG AAG GCG AC-3' and reverse 5'-ATG TTA ACT CAG GGA ATG TGG CA-3'. GAPDH (forward 5'-GCA CCG TCA AGG CTG AGA AC-3' and reverse 5'-ATG GTG GTG AAG ACG CCA GT-3') was used as the reference gene to normalize input RNA.

2.10 Western blot

Western blotting was performed as described previously [11]. Primary antibodies included TGF-β1 (sc-130348, 1:200), Smad3 (sc-101154, 1:250), phospho-Smad3 (Ser423/425, sc-517575, 1:200), Smad7 (sc-365846, 1:200), AT1R (sc-390579, 1:200), collagen I (sc-293182, 1:250), collagen III (sc-271249, 1:250), and β-actin (sc-47778, 1:500) from Santa Cruz Biotechnology (Dallas, TX, USA). Phospho-Smad2 (Ser465/467, #3108) and Smad4 (#46535) antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA) and used at a 1:200 dilution. Secondary antibodies (Cell Signaling Technology) included horseradish peroxidase-labeled goat anti-rabbit and goat anti-mouse IgG, diluted 1:1000-1:2000 in 0.2% TBS-T and 1% skim milk. Protein bands were visualized using ECL Plus (Amersham, Arlington Heights, IL, USA). Band intensities were quantified using ImageJ software (NIH, Bethesda, MD, USA). Target protein levels were normalized to the corresponding loading control shown in each blot (GAPDH or β-actin for total proteins; total Smad2 or total Smad3 for phosphorylated Smad ratios). All Western blot experiments were performed with three independent biological replicates (n=3 per group).

2.11 Measurement of myocardial inflammatory cytokines

LV homogenates were used to measure IL-6 and TNF-α levels using commercial ELISA kits according to the manufacturers' instructions. Cytokine concentrations are presented as pg/mL.

2.12 Statistical analysis

Statistical analyses were conducted using SPSS 25.0 software. All data were expressed as mean ± standard deviation (SD). Normality of data distribution was assessed using the Shapiro-Wilk test. Homogeneity of variances was evaluated using Levene's test. For data meeting normality and homogeneity assumptions, one-way analysis of variance (ANOVA) was performed to compare mean values among the four groups, followed by Tukey's post hoc test for multiple comparisons. For data that did not meet normality assumptions, the Kruskal-Wallis nonparametric test was used, followed by Dunn's post hoc test with Bonferroni correction for multiple comparisons. A value of $P < 0.05$ was considered statistically significant. Sample size (n=20 per group initially) was determined using G*Power software (version 3.1.9.7), assuming an effect size of 0.5 for the primary outcomes (collagen volume fraction and ejection fraction), $\alpha = 0.05$, and power $(1-\beta) = 0.80$. The calculation indicated a minimum of 16 animals per group; we included 20 per group to account for potential mortality and exclusions.

3. Results

3.1 Characteristics and post-infarct survival

All animals exhibited good tolerance to the ET regimen and survived the post-

myocardial infarction period in the MIex groups. Only animals with an infarct size exceeding 25% were included in the analysis. After exclusions (8 rats with infarct size <25% and 4 rats that died during the experimental period), the final numbers analyzed were: Sham, n=20; MI-Sedentary, n=17; $MIex_1$, n=19; $MIex_3$, n=18. Four rats died during the experimental period (2 in MI-Sedentary, 1 in $MIex_1$, 1 in $MIex_3$), all within the first 7 days post-MI due to acute heart failure or arrhythmia. The attributes of these animals are presented in Table 1. Results indicate that there are no differences in infarct size or body weight across the groups. The heart weight (HW) and the heart weight to body weight ratio (HW/BW) were elevated in all MI groups compared to the sham group. Compared with MI-Sedentary rats, $MIex_1$ rats showed a reduction in HW and HW/BW, whereas $MIex_3$ rats showed a more pronounced decline.

3.2 Effects of exercise on cardiac function

Our analysis of cardiac function at 12 weeks after surgery is presented in Table 2 and Figure 2. Compared with sham rats, MI-sedentary rats showed reduced left ventricular systolic pressure (LVSP) and positive and negative maximal values of the instantaneous first derivative of left ventricular pressure (±dP/dtmax), whereas left ventricular end-diastolic pressure (LVEDP) was elevated, indicating systolic and diastolic dysfunction. ET significantly improved LVSP and ±dP/dtmax and reduced LVEDP in MIex rats.

Representative M-mode echocardiograms are shown in Figure 2A. MI-sedentary rats had enlarged LV end-systolic (LVESd) and end-diastolic (LVEDd) diameters, along with reduced ejection fraction (EF) and fractional shortening (FS), compared with sham

rats (Fig. 2B-E). ET reduced LV diameters and improved EF and FS, with greater effects in the MIex3 group than in the MIex1 group. Myocardial IL-6 and TNF-α levels were also increased after MI and attenuated by ET, especially after 12 weeks of training (Fig. 2F, G). These data indicate that ET preserved post-MI cardiac function and reduced the inflammatory response.

3.3 Effects of exercise on Ang II levels and cardiac fibrosis

Myocardial fibrosis (MF) was assessed by gross pathology, Masson's trichrome stain, collagen volume fraction, and collagen type (I and III) (Figure 3). There was substantial MF following myocardial infarction (MI) (Table 1).

The left ventricular free wall of MI-SED was a pale color with a sharply defined demarcation indicative of replacement fibrosis, where the dense collagen had replaced the necrotic cardiomyocytes (Figures 3A and 3B). The shape of the epicardial border of the infarct would be conical, with the point of the cone at the epicardial surface. The collagen fibers were densely packed and highly organized, forming a well-organized scar with very few remaining myocytes.

ET reduced MF, as determined by a decrease in collagen volume fraction; however, a greater extent of ET (12 weeks) reduced MF to a greater degree (Figure 3D). In both MIex1 and MAR-ex3, the amount of collagen deposited was lower than in MI-SED and contained preserved bundles of myocytes at the infarct border zone, and collagen remnants that were loose and immature compared to MI-SED, while containing viable myocardial tissue between the collagen. In all groups, by 12 weeks, no hemorrhage was

observed, indicating complete healing of the acute injury.

MI caused a significant increase in collagen type I and III expression; however, expression of collagen I/III was significantly decreased in MIex1 following 4 weeks of exercise and in MIex3 after 12 weeks of exercise (Figure 3C and 3E).

Compared with the sham group, MI rats had a significantly greater amount of Ang II in their LV (Table 1). MIex3 showed a significant reduction in Ang II after 12 weeks of ET in the post-infarct LV; however, MIex1 showed a lower Ang II level after 4 weeks of ET, but this difference was not significant.

3.4 Effects of exercise on AT1R/TGF-β1 expression

To evaluate the effect of ET on the upstream AT1R/TGF- β 1 pathway after MI, we performed qRT-PCR and Western blot analyses. AT1R expression was increased in MI-sedentary rats and reduced by ET, with the greatest decrease after 12 weeks of training (Fig. 4A, C). This reduction was accompanied by lower collagen I/III deposition, suggesting that downregulation of cardiac AT1R may be associated with exercise-related improvement in post-MI cardiac fibrosis.

Because Ang II-induced AT1R activation can upregulate TGF-β1 during post-infarct cardiac fibrosis, we next assessed TGF-β1. TGF-β1 protein expression was markedly increased in LV tissues of MI-sedentary rats and was attenuated by ET, particularly in the MIex3 group (Fig. 4B, D).

3.5 Effects of exercise on downstream Smad phosphorylation

We next examined downstream Smad activation. MI increased phosphorylation of

Smad2 and Smad3 in LV tissue, whereas ET attenuated p-Smad2/Smad2 and p-Smad3/Smad3 ratios (Fig. 4E-H). The reduction was more pronounced in the MIex3 group than in the MIex1 group. These data suggest that ET is associated with lower activity of the TGF-β1/Smad pathway in MI-induced cardiac fibrosis.

4. Discussion

This research yielded four key conclusions: (1) Exercise training (ET) improved preservation of cardiac functioning and reduced post-MI inflammation; (2) ET reduced collagen deposited and the ratio of proteins I/III in the damaged heart following MI; (3) ET reduced the expression of Ang II, AT1R, TGF-β1 and phosphorylated Smad 2/3 activity; and (4) A longer duration of ET provided greater improvements in fibrotic changes to the heart and cardiac function as compared to a shorter duration of ET. Taken together, these conclusions support the notion that prolonged exercise can attenuate myocardial fibrosis and reduce AT1R/TGF-β1/Smad signaling after MI.

In addition, based on what has been reported in the literature, exercise rehabilitation following MI is considered an adjunct treatment to both reperfusion therapy and drug therapy for the purpose of improving post-MI remodeling [15,27]. Studies using experimental designs demonstrate the benefits associated with long-term ET on enhancing the quality of life and decreasing the risk for mortality among individuals with an MI or heart failure [27,28]. Furthermore, the literature provides ample evidence that exercise enhances cardiac performance and reduces maladaptive remodeling in animal models of MI [18,29,30]. Our data are consistent with previous reports showing ET's ability to decrease left ventricular dilation and increase left ventricular contractility

and diastolic function when initiated shortly after MI.

Biopsies from patients and animal models demonstrated that ventricular fibrosis is a significant feature of MI-induced structural remodeling [31, 32]. The extensive accumulation of collagen tissue in the extracellular matrix results in increased ventricular stiffness and impaired mechanical properties [3, 4]. We observed that MI induced profound changes in the expression of collagens and fibrogenic molecules in post-infarct hearts. Previous studies have reported that post-MI exercise can ameliorate collagen deposition, inflammatory response, scar thinning, and resultant cardiac dysfunction in rats [18, 33]. In this study, ET reduced MI-induced collagen accumulation, which was accompanied by improved cardiac function. Moreover, extended ET manifested with greater anti-fibrotic effects. Thus, exercise may preserve post-MI cardiac function by attenuating myocardial fibrosis, an effect that may be enhanced by prolonged exercise.

Ang II plays a vital role in the development of cardiac fibrosis and remodeling, primarily through the AT1R, as confirmed in our previous study [6, 11]. Clinical studies have demonstrated that inhibition of cardiac RAAS by angiotensin II receptor blockers improves LV function, prevents ventricular remodeling, and prolongs survival in patients with MI and heart failure [34, 35]. Antagonism of AT_1R could also attenuate MI-induced myocardial fibrosis in rat models [36, 37]. Interestingly, exercise has been reported to decrease circulating Ang II levels, reduce local AT1R expression, and ameliorate cardiac dysfunction in failing, infarcted, and aging hearts [22, 38, 39].

Here, local Ang II and AT1R expression were increased in MI hearts, consistent

with previous findings. ET reduced their expression, and the reduction was more pronounced after longer training. The decrease in Ang II/AT1R signaling was accompanied by improved cardiac function and reduced myocardial fibrosis. Therefore, our data indicate that exercise-induced cardioprotective effects in post-MI hearts may be associated with downregulation of the Ang II/AT1R pathway. We further explored downstream molecular changes of AT1R in response to exercise.

Ang II/AT_1R-mediated activation of TGF-β_1/Smad signaling is a well-established pathway that promotes cardiac fibrosis in heart failure and MI. However, little is known about the changes in molecules associated with TGF-β_1 signaling in exercise-mediated effects. We previously confirmed that the AT_1R-specific degradation of Smad7 decreased the inhibitory feedback regulation of TGF-β_1/Smad3/Smad4 signaling, thereby promoting collagen accumulation in atrial fibrosis and remodeling [10, 11]. It is also reported that decreased Smad7 expression, by activating TGF-β1/Smad2/Smad3 signaling, aggravated cardiac fibrosis following MI [40].

Potential role of BDNF and NO: Emerging evidence suggests that exercise-induced cardioprotection may also involve brain-derived neurotrophic factor (BDNF) and nitric oxide (NO). Exercise training is known to enhance BDNF expression in the cardiovascular system, and BDNF has been reported to attenuate cardiac fibrosis by suppressing TGF-β1/Smad signaling [41,42]. Similarly, exercise increases endothelial NO synthase (eNOS) activity and NO bioavailability [43]. NO can inhibit TGF-β1-induced fibroblast activation and collagen synthesis through cGMP-dependent pathways, and may also upregulate Smad7 expression, thereby enhancing the negative feedback

loop that limits TGF-β1/Smad3-mediated fibrosis [44,45]. While our study did not directly measure BDNF or NO levels, it is plausible that these exercise-induced mediators contribute to the observed downregulation of AT1R/TGF-β1/Smad signaling and upregulation of Smad7. Future studies should investigate whether BDNF and NO directly enhance Smad7 expression and whether inhibition of these pathways abrogates the anti-fibrotic effects of exercise post-MI.

In this study, MI-induced activation of the Ang II/AT1R/TGF-β1 pathway was accompanied by increased phosphorylation of Smad2 and Smad3 and by increased collagen I/III expression in the post-infarct myocardium. ET attenuated Smad2/3 phosphorylation and collagen deposition, with stronger effects after 12 weeks of ET. Thus, the exercise-related reduction in cardiac fibrosis and preservation of cardiac function may be, at least in part, associated with inhibition of TGF-β1/Smad signaling. Direct pathway interventions are still needed to establish causality.

5. Limitations

There are several limitations of this study. First, the findings of this study are correlative, and direct intervention strategies (i.e., an AT1R blocker, a TGF-beta inhibitor, or a Smad modulator) must be performed to test causality of the observed correlation. Second, only one exercise intensity has been studied (lower-intensity exercise); the effects observed may be dependent on intensity. Third, the gradual development of the changes seen over time suggests that an effect of time exists, though neither the short-term nor long-term effects of exercise have been evaluated. Fourth, how exercise regulates the feedback of

inhibitory Smads and inflammatory signaling is currently unknown. Fifth, the sample size of this study was limited; thus, the findings need to be replicated in a larger sample and across different animal species, including humans.

6. Conclusions

This research provides evidence for the cardioprotective effect of exercise training (ET) on maintaining cardiac systolic function and reducing myocardial fibrosis following myocardial infarction (MI). The decrease in the signaling pathway Ang II/AT1R/TGF-β1/Smad observed across both ET groups may indicate that long-term ET exerts cardioprotective effects. Long-term ET (12 weeks) provided greater anti-fibrotic and functional improvements than short-term ET (4 weeks), suggesting a time-dependent effect of ET. These preliminary findings support the efficacy of ET-based rehabilitation after MI but require clinical validation before direct application in human patients. Studies investigating direct pathway interventions (e.g., AT1R blockade, TGF-beta inhibition, or Smad modulation) will be needed to establish a cause-and-effect relationship.

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Author Contributions:

Author Contributions: Conceptualization: Yusen Ou, Xueqian Huang; Investigation: Yusen Ou, Xueqian Huang, Jialin Lu; Data curation: Yusen Ou, Xueqian Huang, Jialin Lu; Writing - review and editing: Moussa Ide Nasser, Xuyu He; Funding acquisition:

Xuyu He.

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Availability of data and material: The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethics approval:

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Guangdong Provincial People's Hospital of Guangdong Academy of Medical Sciences (Approval No. KY2020-491-02). The approval was granted on August 15, 2020, under the title 'Mechanisms of Exercise-Induced Cardioprotection After Myocardial Infarction: Role of the AT1R/TGF-β1/Smad Pathway.'

Clinical trial number: not applicable

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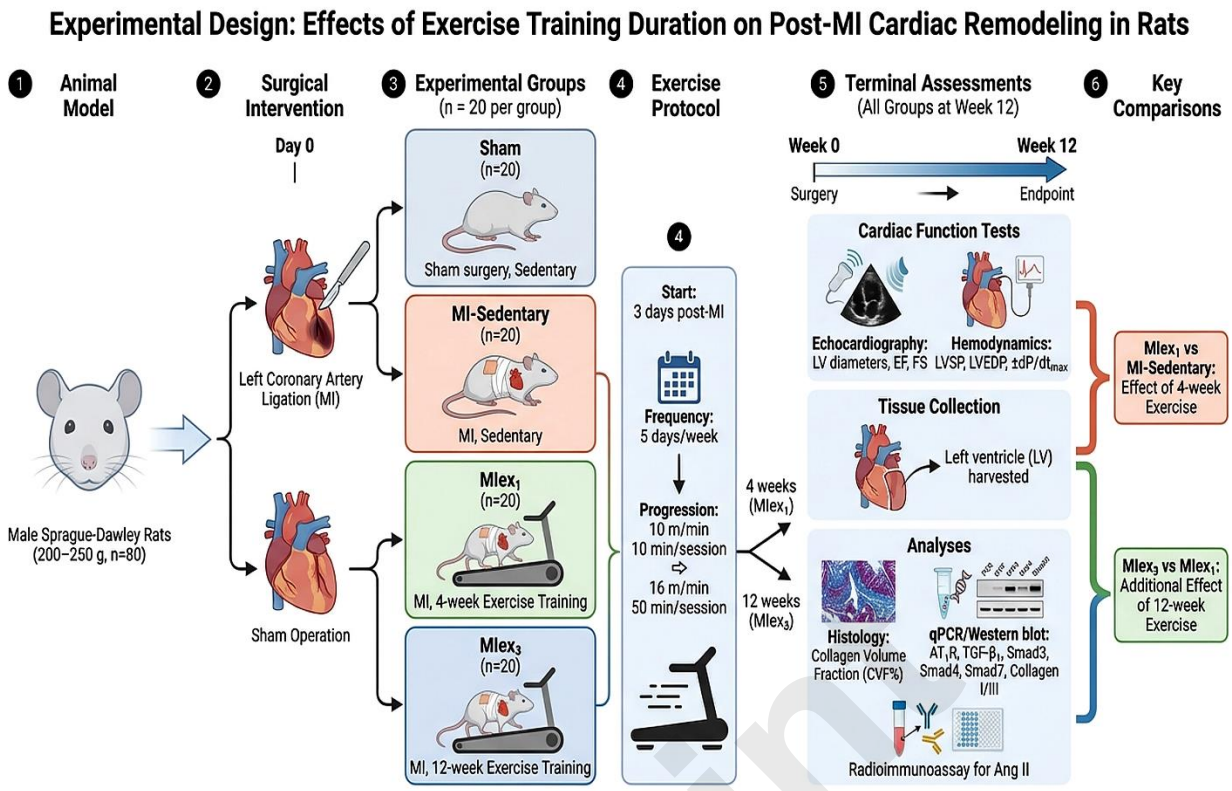


Figure 1. Experimental workflow. Male Sprague-Dawley rats (n=80) underwent left coronary artery ligation to induce myocardial infarction (MI) or sham surgery. Animals were randomly assigned to four groups (n=20 each): sham, MI-sedentary, MI with 4-week exercise training (MIex1), and MI with 12-week exercise training (MIex3). ET was initiated 3 days post-MI on a rodent treadmill (5 days/week; gradually increasing from 10 m/min for 10 min to 16 m/min for 50 min per session). At the 12-week endpoint, echocardiography and hemodynamic measurements were performed, followed by LV tissue collection for histology, collagen quantification, qRT-PCR/Western blotting, and Ang II radioimmunoassay.

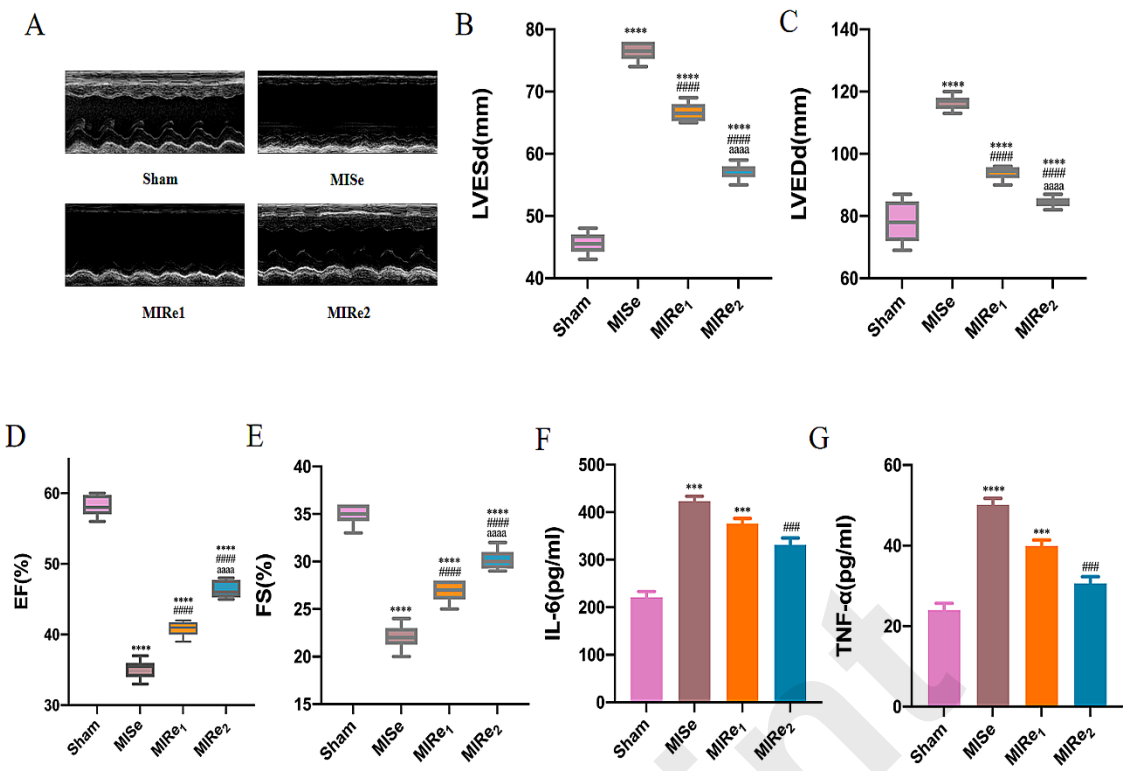


Figure 2. Effects of exercise on cardiac function and inflammatory markers after MI. (A) Representative M-mode echocardiographic images. Quantitative analysis of (B) LV end-systolic diameter (LVESd), (C) LV end-diastolic diameter (LVEDd), (D) ejection fraction (EF), and (E) fractional shortening (FS). Myocardial inflammatory markers are shown as (F) IL-6 and (G) TNF-α. In the figure labels, MISe/MI indicates MI-sedentary; MIRe1/Ren-1 indicates MIex1; and MIRe2/Ren-2 indicates MIex3. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 vs. Sham; #P < 0.05, ###P < 0.001, ####P < 0.0001 vs. MI-sedentary.

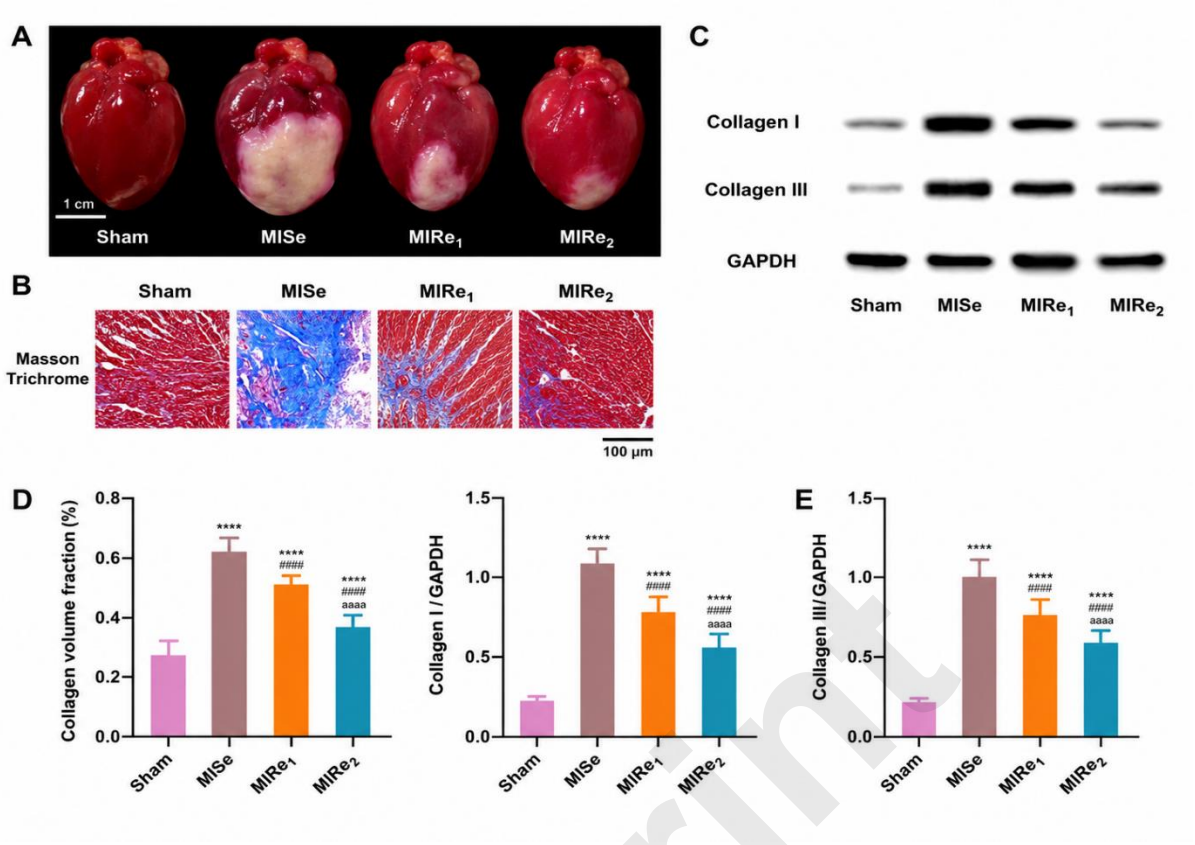


Figure 3. Effects of exercise on cardiac collagen deposition after MI.

(A) Gross morphology of representative hearts. (B) Masson's trichrome staining of LV sections; myocardium is stained red and collagen is stained blue. (C) Representative Western blots for collagen I and collagen III. (D) Quantification of collagen volume fraction. (E) Quantification of collagen I and collagen III protein levels normalized to GAPDH. In the figure labels, MISe indicates MI-sedentary, MIRe1 indicates MIex1, and MIRe2 indicates MIex3. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 vs. Sham; #P < 0.05, ####P < 0.0001 vs. MI-sedentary.

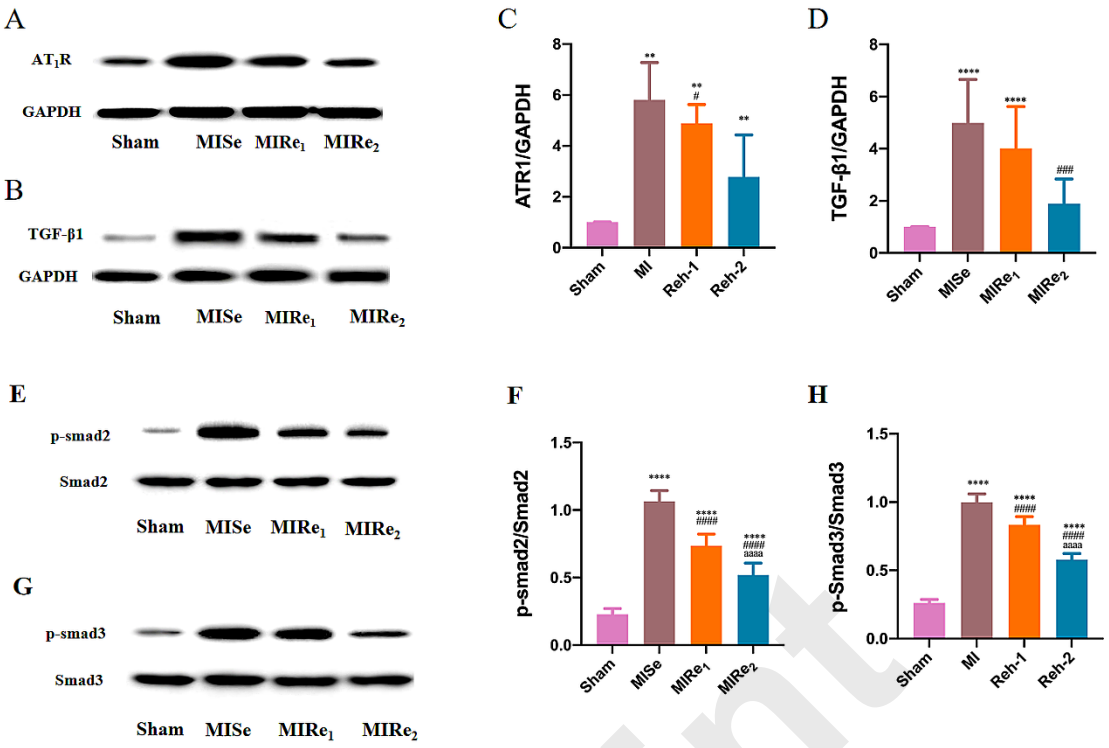


Figure 4. Effects of exercise on AT1R/TGF-β1/Smad signaling after MI.

Representative Western blots for (A) AT1R and (B) TGF-β1, with quantitative analysis of (C) AT1R/GAPDH and (D) TGF-β1/GAPDH. Representative blots and quantification are also shown for (E,F) p-Smad2/Smad2 and (G,H) p-Smad3/Smad3. In the figure labels, MISe/MI indicates MI-sedentary; MIRe1/Ren-1 indicates MIex1; and MIRe2/Ren-2 indicates MIex3. *P < 0.05, **P < 0.01, ****P < 0.0001 vs. Sham; #P < 0.05, ###P < 0.001, ####P < 0.0001 vs. MI-sedentary.

Experimental Design: Effects of Exercise Training Duration on Post-MI Cardiac Remodeling in Rats

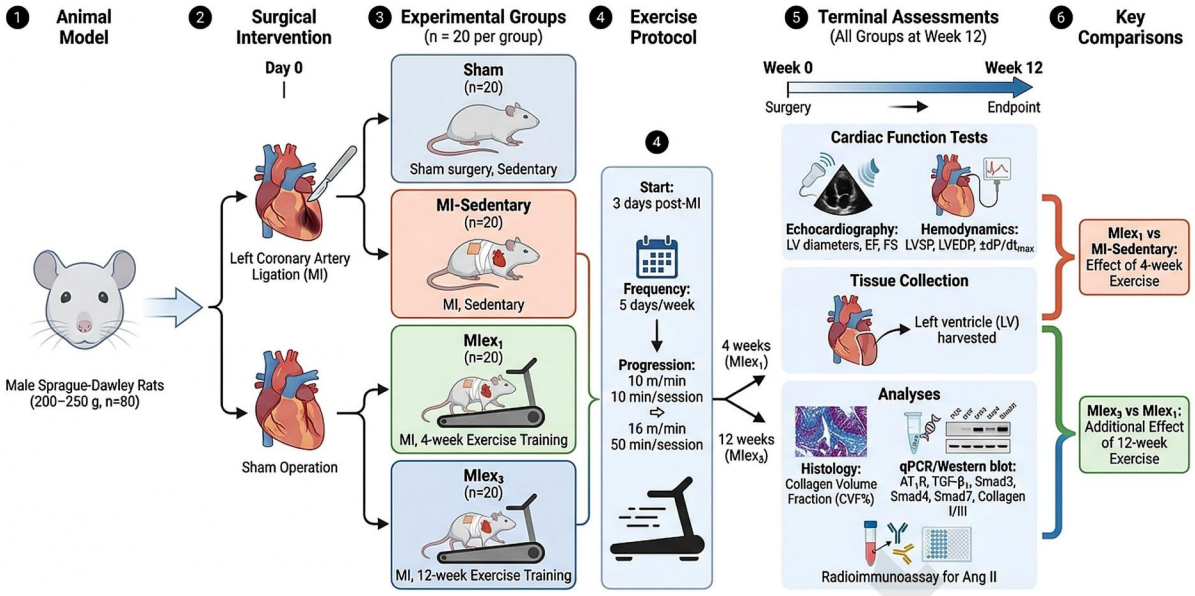


Table 1: Table 1. Characteristics of Experimental Rats

Parameter	Sham	MI-Sedentary	MIex$_1$	MIex$_3$
Infarct size (%)	–	33.53 ± 1.23	31.38 ± 1.63	34.93 ± 1.45
Body weight (g)	361.2 ± 8.2	366.5 ± 7.8	363.7 ± 6.9	360.8 ± 8.1
Heart weight (g)	3.26 ± 0.21	3.82 ± 0.13*	3.80 ± 0.26*	3.53 ± 0.26*#
Ht Wt / BW (g/kg)	9.03 ± 0.10	10.39 ± 0.11*	10.38 ± 0.08*	9.67 ± 0.13*#
Systolic aortic pressure (mmHg)	114.26 ± 1.39	100.31 ± 1.08*	101.63 ± 1.26*	101.95 ± 1.47*
Diastolic aortic pressure (mmHg)	84.27 ± 0.58	72.46 ± 0.83*	73.07 ± 1.17*	74.64 ± 1.33*
Ang II (pg/mg)	142.5 ± 28.4	234.8 ± 30.6*	223.5 ± 27.9*	186.2 ± 29.2*#
Hydroxyproline (μg/mg)	4.79 ± 0.15	7.24 ± 0.11*	7.02 ± 0.09*	5.86 ± 0.13*#

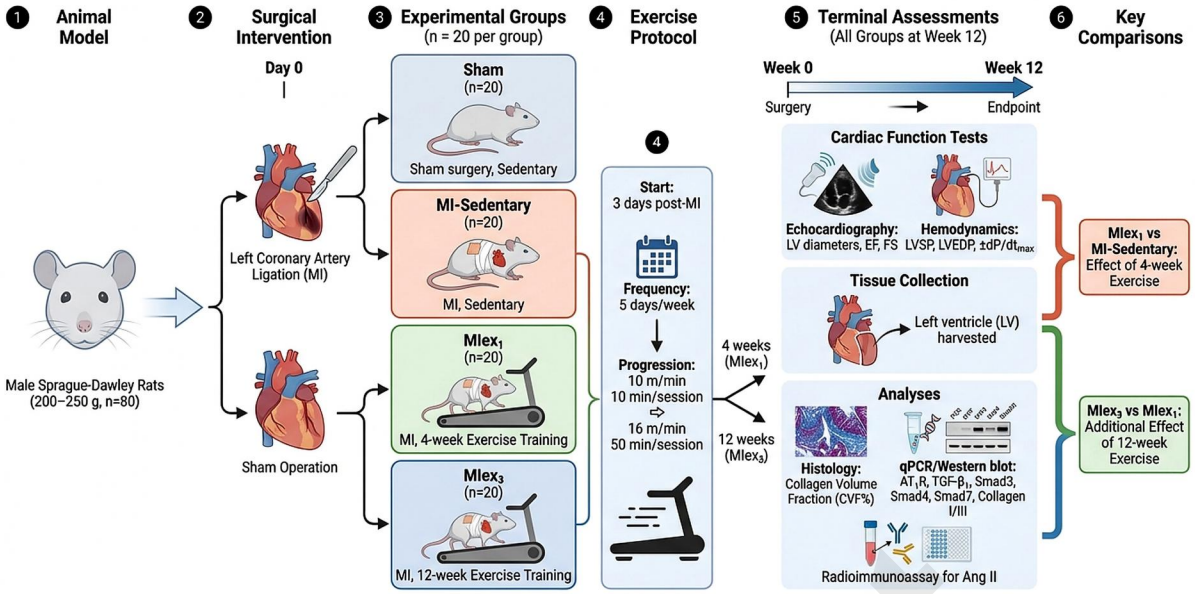
Values are means ± SE. MI: myocardial infarction; MIex: MI plus exercise groups; Ht Wt/BW: ratio of heart weight to body weight. *P ＜0.05 vs. Sham; #P ＜ 0.05 vs. MI.

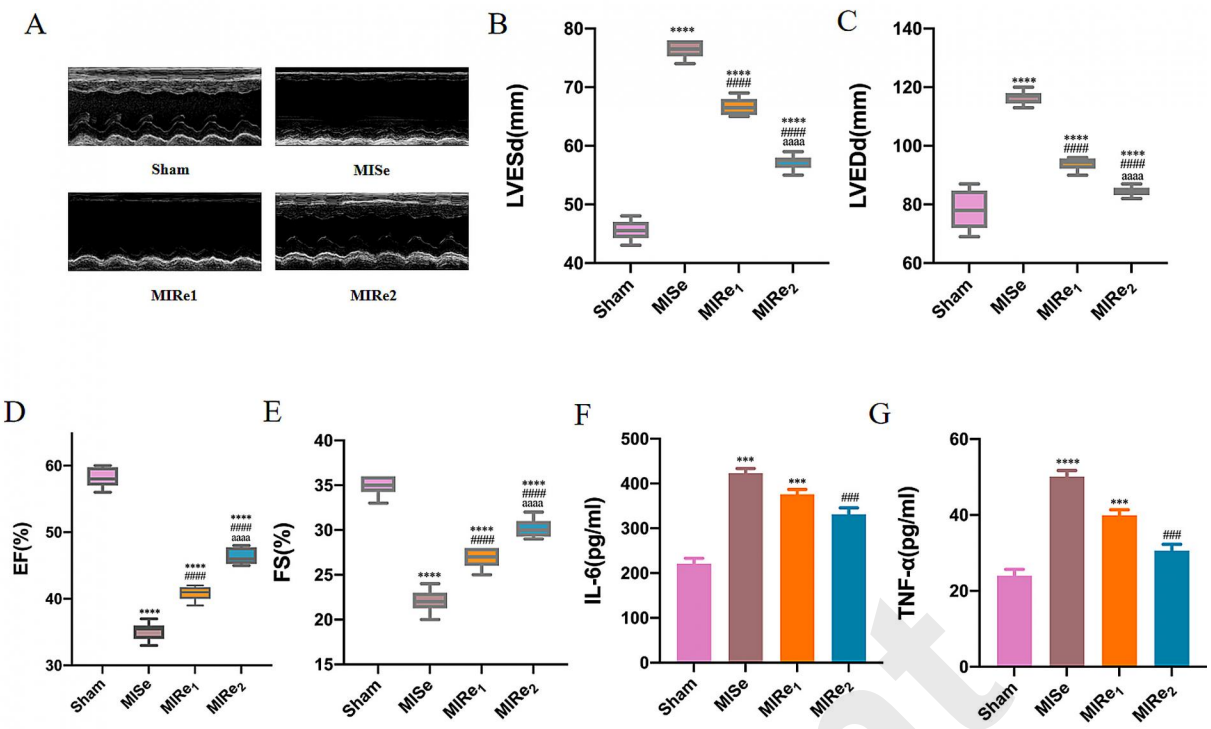
Table 2. Echocardiographic and Hemodynamic Parameters of Cardiac Function

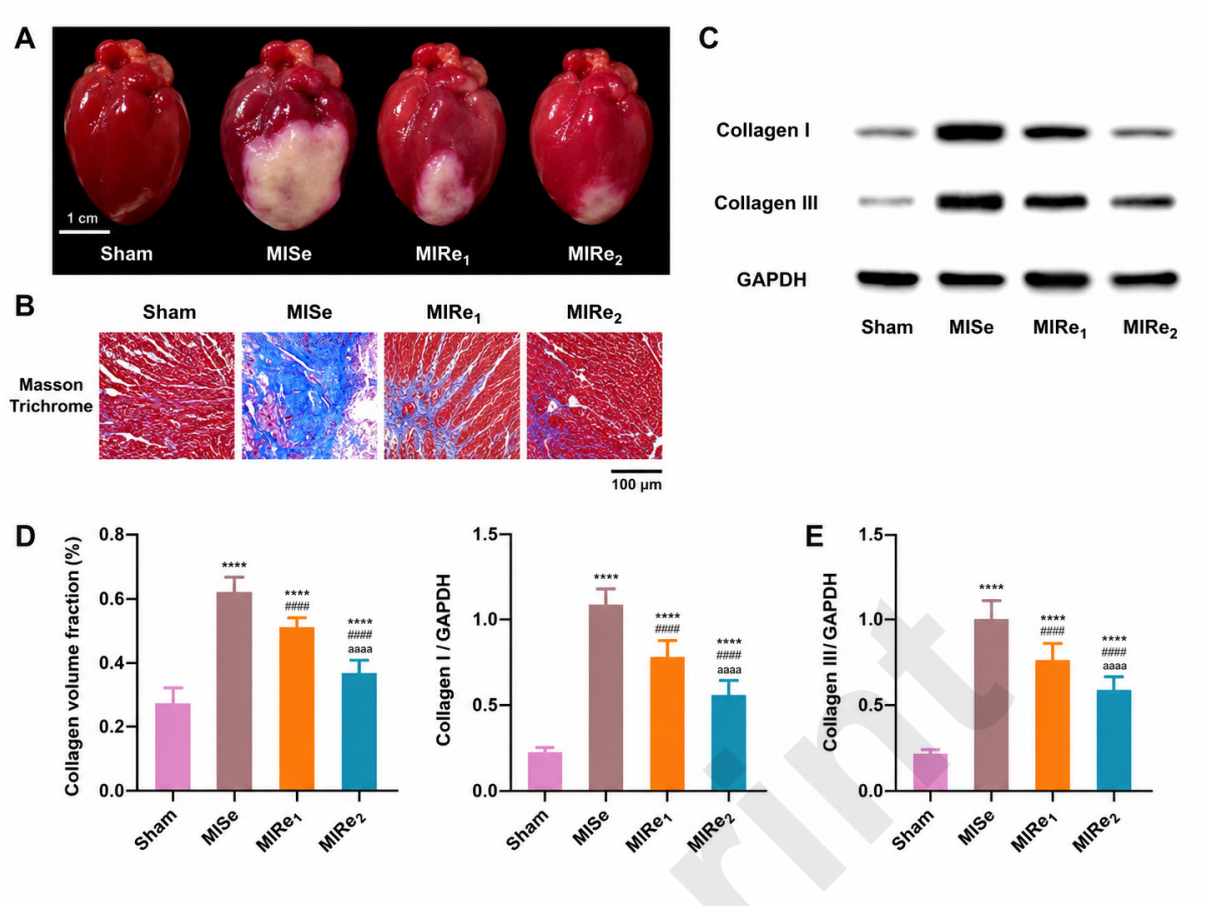
Parameter	Sham	MI-Sedentary	MIex$_1$	MIex$_3$
Heart rate (bpm)	298 ± 22	304 ± 16	292 ± 19	289 ± 20
LVSP (mmHg)	104.73 ± 2.02	90.45 ± 3.16*	93.84 ± 3.46*	99.74 ± 3.38*#
LVEDP (mmHg)	4.35 ± 0.48	9.85 ± 0.43*	8.86 ± 0.37*	7.64 ± 0.64*#
LV +dP/dtmax (mmHg/s)	6422 ± 238.8	3978 ± 339.3*	4183 ± 325.3*	5048 ± 353.5*#
LV –dP/dtmax (mmHg/s)	6502 ± 379.4	3238 ± 336.7*	4394 ± 297.3*	5136 ± 352.4*#
LVEDd (mm)	7.35 ± 0.21	12.03 ± 0.17*	11.89 ± 0.15*	11.80 ± 0.20*
LVESd (mm)	4.22 ± 0.16	11.10 ± 0.18*	9.94 ± 0.18*	9.23 ± 0.18*#
Ejection fraction (%)	0.86 ± 0.10	0.47 ± 0.08*	0.66 ± 0.11*#	0.72 ± 0.12*#
Fractional shortening (%)	47.12 ± 1.08	26.17 ± 1.24*	28.08 ± 1.24*	28.17 ± 1.24*

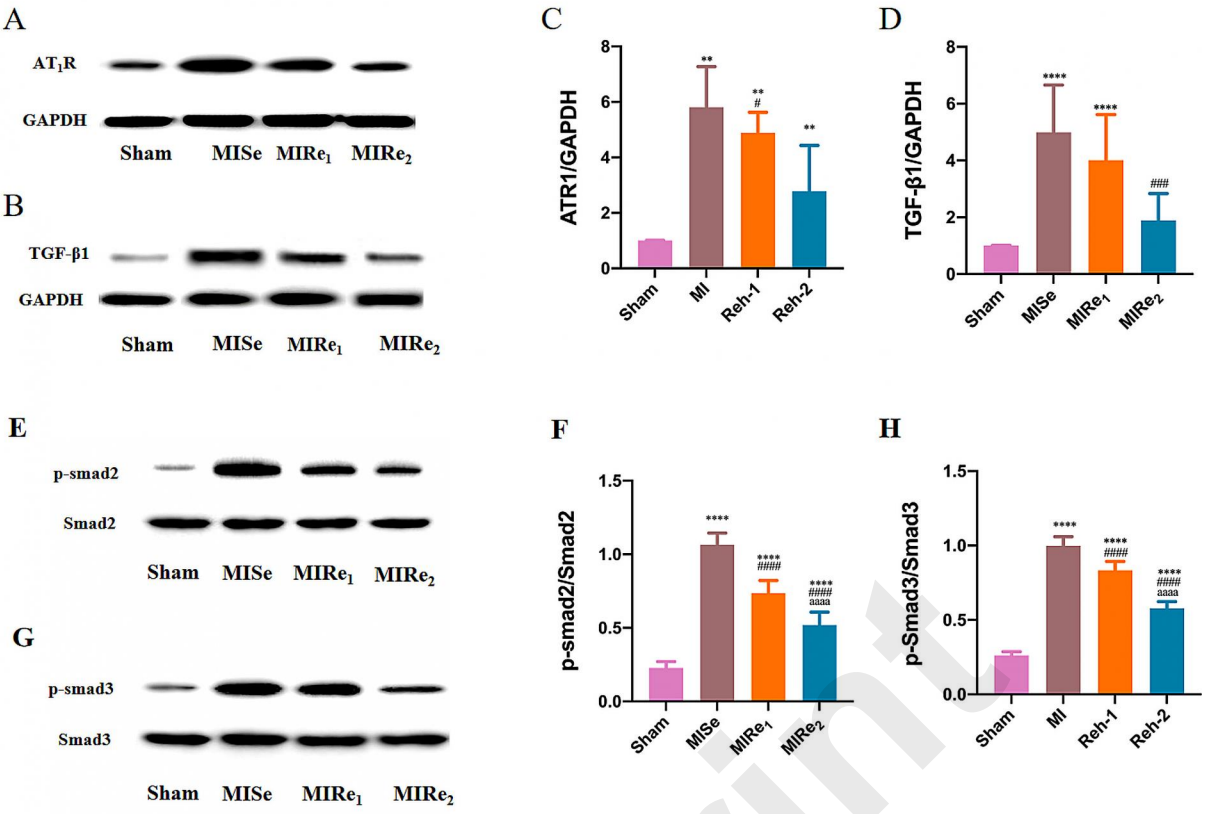
Values are means ± SE. MI: myocardial infarction; MIex: MI plus exercise groups; LVSP: left ventricular systolic pressure; LVEDP: left ventricular end diastolic pressure; ± dP/dtmax: positive and negative maximal values of the instantaneous first derivative of left ventricular pressure; LVEDd: left ventricular end diastolic diameter; LVESd: left ventricular end systolic diameter. *P < 0.05 vs. Sham; #P <0.05 vs. MI.

Experimental Design: Effects of Exercise Training Duration on Post-MI Cardiac Remodeling in Rats




A
Sham
MISe
MIRe1
MIRe2
B
LVESd(mm)
C
LVEDd(mm)
D
EF(%)
E
FS(%)
F
IL-6(pg/ml)
G
TNF-α(pg/ml)
Sham
MISe
MIRe1
MIRe2


A
1 cm
Sham
MISe
MIRe1
MIRe2
B
Sham
MISe
MIRe1
MIRe2
Masson Trichrome
100 µm
C
Collagen I
Collagen III
GAPDH
Sham
MISe
MIRe1
MIRe2
D
Collagen volume fraction (%)
0.0
0.2
0.4
0.6
0.8
****
****
####
****
####
aaaa
Sham
MISe
MIRe1
MIRe2
Collagen I / GAPDH
0.0
0.5
1.0
1.5
****
****
####
****
####
aaaa
Sham
MISe
MIRe1
MIRe2
E
Collagen III/ GAPDH
0.0
0.5
1.0
1.5
****
****
####
****
####
aaaa
Sham
MISe
MIRe1
MIRe2


A
AT1R
GAPDH
Sham
MISe
MIRe1
MIRe2
B
TGF-β1
GAPDH
Sham
MISe
MIRe1
MIRe2
C
ATR1/GAPDH
Sham
MI
Reh-1
Reh-2
D
TGF-β1/GAPDH
Sham
MISe
MIRe1
MIRe2
E
p-smad2
Smad2
Sham
MISe
MIRe1
MIRe2
F
p-smad2/Smad2
Sham
MISe
MIRe1
MIRe2
G
p-smad3
Smad3
Sham
MISe
MIRe1
MIRe2
H
p-Smad3/Smad3
Sham
MI
Reh-1
Reh-2