

Association of AT1R-mediated TGF- β 1/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- β 1/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 ET (Mlex1), and MI with 12-week ET (Mlex3). ET began three days after coronary artery ligation. After 12 weeks, echocardiography and haemodynamic measurements were performed, and left ventricular (LV) tissue was collected for histological and molecular analyses.

Results: Compared with MI-sedentary rats, Mlex1 rats showed increased LV systolic pressure, $\pm$ 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- β 1, 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- β 1/Smad signalling may be associated with these cardioprotective effects.

Key words: exercise training, myocardial infarction, cardiac fibrosis, TGF- β 1, SMAD signalling, angiotensin II type 1 receptor, rat model, cardiac pathology, echocardiography, systolic function.

Introduction

Despite modern reperfusion and guideline-directed drugs, post-myocardial infarction (MI) left ventricular (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-re-modeling therapies [2, 3].

Post-MI remodeling is primarily driven by fibrosis, characterised 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 angiotensin II type 1 receptor (AT1R), which activate the TGF- β 1/SMAD cascade [5–7]. Ligand binding to the TGF- β 1 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 stabilises 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 normalising 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 stabilised mitochondrial biogenesis [18–20]. Nonetheless, there is a paucity of data on the molecular signalling mechanisms underlying ET-induced cardioprotection in post-MI hearts.

The present study, therefore, aimed to determine the following: (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 signalling; and (3) whether prolonged ET demonstrates greater benefits for myocardial fibrosis and cardiac function than short-term ET.

Material 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.

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.

Animals and MI model

Male Sprague-Dawley rats weighing 200–250 g were obtained from the Experimental Animal Centre 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].

Experimental groups and exercise protocol

As illustrated in Figure 1, a total of 80 rats were randomly assigned to four groups: Sham-operation control (Sham, $n = 20$), MI-Sedentary (MI, $n = 20$), MI plus 4-week ET (Mlex₁, $n = 20$), and MI plus 12-week ET (Mlex₃, $n = 20$). The Mlex₁ 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 Mlex₃ group underwent exercise training continuously for all 12 weeks.

According to previous studies [17, 22], ET was initiated in all Mlex 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 at a 5° incline for 10 min per session, then gradually increased to 16 m/min and 50 minutes per session and maintained at this level throughout the experiment.

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].

Haemodynamic measurements

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

Radioimmunoassay of Ang II accumulation

LV tissues (50 mg) were homogenised in cold acetic acid, centrifuged, and processed according

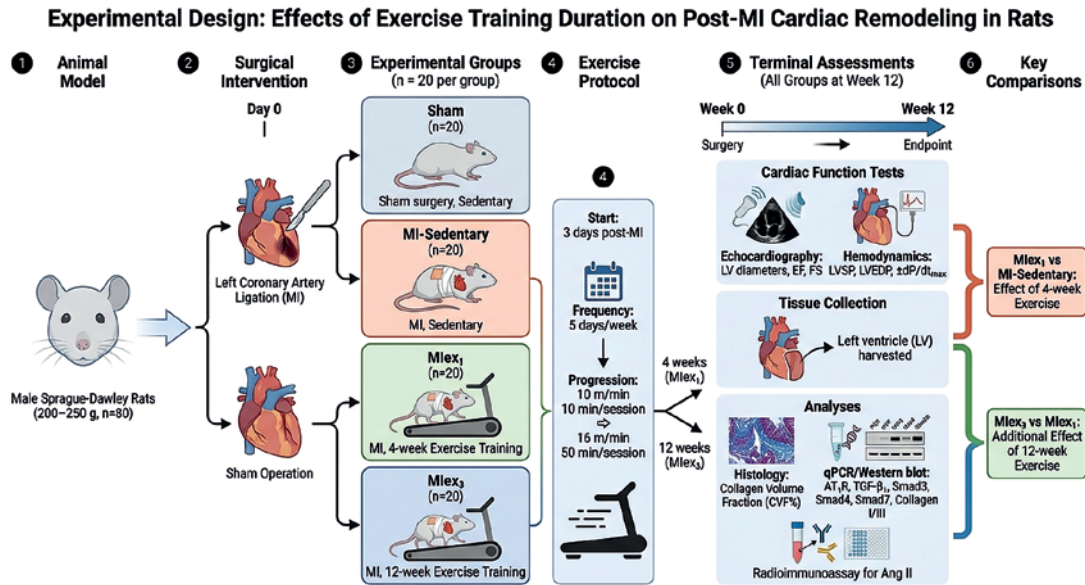


Figure 1. Experimental workflow. Male Sprague-Dawley rats ($n = 80$) underwent left coronary artery ligation to induce myocardial infarction (MI) or sham surgery. Animals were randomly assigned to four groups ($n = 20$ each): sham, MI-sedentary, MI with 4-week exercise training (Mlex₁), and MI with 12-week exercise training (Mlex₃). 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

to the instructions of the 125I-CAMP radioimmunoassay kit (Medical College of Sun Yat-sen University, Guangzhou, China) to quantify Ang II accumulation.

Tissue collection

After completion of haemodynamic measurements, rats were euthanised 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 RNeasy lysis buffer (Qiagen) at -80°C for RNA extraction.

Masson trichrome staining and collagen volume fraction

LV tissues were fixed in 10% neutral buffered formalin for 24 h, dehydrated through a graded ethanol series (70%, 80%, 95%, 100%), cleared in xylene, and embedded in paraffin wax (melting point $56-58^{\circ}\text{C}$) 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 previ-

ously described [25] using a commercial kit (Sigma-Aldrich, HT15). Briefly, sections were stained with Weigert's iron haematoxylin for 10 min, Biebrich scarlet acid fuchsin for 5 min, phosphomolybdic-phosphotungstic acid for 10 min, and aniline blue for 5 min, with washes between steps.

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 the 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- β 1: 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 AA-3' and reverse 5'-GAT GGA GAA ACC AGG AA 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 normalise input RNA.

Western blot

Western blotting was performed as described previously [11]. Primary antibodies included TGF- β 1 (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-labelled goat anti-rabbit and goat anti-mouse IgG, diluted 1 : 1000–1 : 2000 in 0.2% TBS-T and 1% skimmed milk. Protein bands were visualised using ECL Plus (Amersham, Arlington Heights, IL, USA). Band intensities were quantified using ImageJ software (NIH, Bethesda, MD, USA). Target protein levels were normalised 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).

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.

Statistical analysis

Statistical analyses were conducted using SPSS 25.0 software. All data were expressed as mean $\pm$ 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 ho-

mogeneity 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. The 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.

Results

Characteristics and post-infarct survival

All animals exhibited good tolerance to the ET regimen and survived the post-myocardial infarction period in the Mlex 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 analysed were: Sham, $n = 20$; MI-Sedentary, $n = 17$; Mlex₁, $n = 19$; and Mlex₃, $n = 18$. Four rats died during the experimental period (2 in MI-Sedentary, 1 in Mlex₁, and 1 in Mlex₃), all within the first 7 days post-MI due to acute heart failure or arrhythmia. The attributes of these animals are presented in Table I. 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, Mlex₁ rats showed a reduction in HW and HW/BW, whereas Mlex₃ rats showed a more pronounced decline.

Table I. Characteristics of experimental rats

Parameter	Sham	MI-Sedentary	Mlex ₁	Mlex ₃
Infarct size (%)	–	33.53 $\pm$ 1.23	31.38 $\pm$ 1.63	34.93 $\pm$ 1.45
Body weight [g]	361.2 $\pm$ 8.2	366.5 $\pm$ 7.8	363.7 $\pm$ 6.9	360.8 $\pm$ 8.1
Heart weight [g]	3.26 $\pm$ 0.21	3.82 $\pm$ 0.13*	3.80 $\pm$ 0.26*	3.53 $\pm$ 0.26**
Ht Wt/BW [g/kg]	9.03 $\pm$ 0.10	10.39 $\pm$ 0.11*	10.38 $\pm$ 0.08*	9.67 $\pm$ 0.13**
Systolic aortic pressure [mm Hg]	114.26 $\pm$ 1.39	100.31 $\pm$ 1.08*	101.63 $\pm$ 1.26*	101.95 $\pm$ 1.47*
Diastolic aortic pressure [mm Hg]	84.27 $\pm$ 0.58	72.46 $\pm$ 0.83*	73.07 $\pm$ 1.17*	74.64 $\pm$ 1.33*
Ang II [pg/mg]	142.5 $\pm$ 28.4	234.8 $\pm$ 30.6*	223.5 $\pm$ 27.9*	186.2 $\pm$ 29.2**
Hydroxyproline [μ g/mg]	4.79 $\pm$ 0.15	7.24 $\pm$ 0.11*	7.02 $\pm$ 0.09*	5.86 $\pm$ 0.13**

Values are means $\pm$ SE. MI – myocardial infarction, Mlex – 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 II. Echocardiographic and haemodynamic parameters of cardiac function

Parameter	Sham	MI-Sedentary	Mlex ₁	Mlex ₃
Heart rate [bpm]	298 $\pm$ 22	304 $\pm$ 16	292 $\pm$ 19	289 $\pm$ 20
LVSP [mm Hg]	104.73 $\pm$ 2.02	90.45 $\pm$ 3.16*	93.84 $\pm$ 3.46*	99.74 $\pm$ 3.38**
LVEDP [mm Hg]	4.35 $\pm$ 0.48	9.85 $\pm$ 0.43*	8.86 $\pm$ 0.37*	7.64 $\pm$ 0.64**
LV +dP/dtmax [mm Hg/s]	6422 $\pm$ 238.8	3978 $\pm$ 339.3*	4183 $\pm$ 325.3*	5048 $\pm$ 353.5**
LV -dP/dtmax [mm Hg/s]	6502 $\pm$ 379.4	3238 $\pm$ 336.7*	4394 $\pm$ 297.3*	5136 $\pm$ 352.4**
LVEDd [mm]	7.35 $\pm$ 0.21	12.03 $\pm$ 0.17*	11.89 $\pm$ 0.15*	11.80 $\pm$ 0.20*
LVEsd [mm]	4.22 $\pm$ 0.16	11.10 $\pm$ 0.18*	9.94 $\pm$ 0.18*	9.23 $\pm$ 0.18**
Ejection fraction (%)	0.86 $\pm$ 0.10	0.47 $\pm$ 0.08*	0.66 $\pm$ 0.11**	0.72 $\pm$ 0.12**
Fractional shortening (%)	47.12 $\pm$ 1.08	26.17 $\pm$ 1.24*	28.08 $\pm$ 1.24*	28.17 $\pm$ 1.24*

Values are means $\pm$ SE. MI – myocardial infarction, Mlex – MI plus exercise groups, LVSP – left ventricular systolic pressure, LVEDP – left ventricular end diastolic pressure, $\pm$ 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.

Effects of exercise on cardiac function

Our analysis of cardiac function at 12 weeks after surgery is presented in Table II 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 ($\pm$ dP/dtmax), whereas left ventricular end-diastolic pressure (LVEDP) was elevated, indicating systolic and diastolic dysfunction. ET significantly improved LVSP and $\pm$ dP/dtmax, and reduced LVEDP in Mlex rats.

Representative M-mode echocardiograms are shown in Figure 2 A. 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 (Figures 2 B–E). ET reduced LV diameters and improved EF and FS, with greater effects in the Mlex3 group than in the Mlex1

group. Myocardial IL-6 and TNF- α levels were also increased after MI and attenuated by ET, especially after 12 weeks of training (Figures 2 F, G). These data indicate that ET preserved post-MI cardiac function and reduced the inflammatory response.

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 I).

The left ventricular free wall of MI-SED was a pale colour with a sharply defined demarcation indicative of replacement fibrosis, where the dense collagen had replaced the necrotic cardiomyocytes (Figures 3 A, B). The shape of the epicardial border of the infarct would be conical, with the point of the cone at the epicardial surface. The collagen fibres were densely

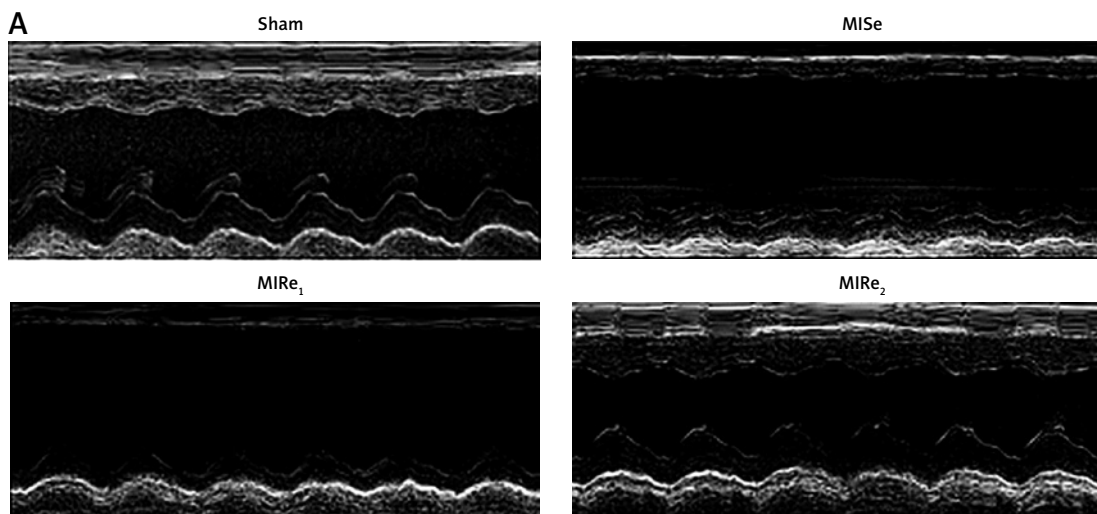


Figure 2. Effects of exercise on cardiac function and inflammatory markers after MI. **A** – Representative M-mode echocardiographic images

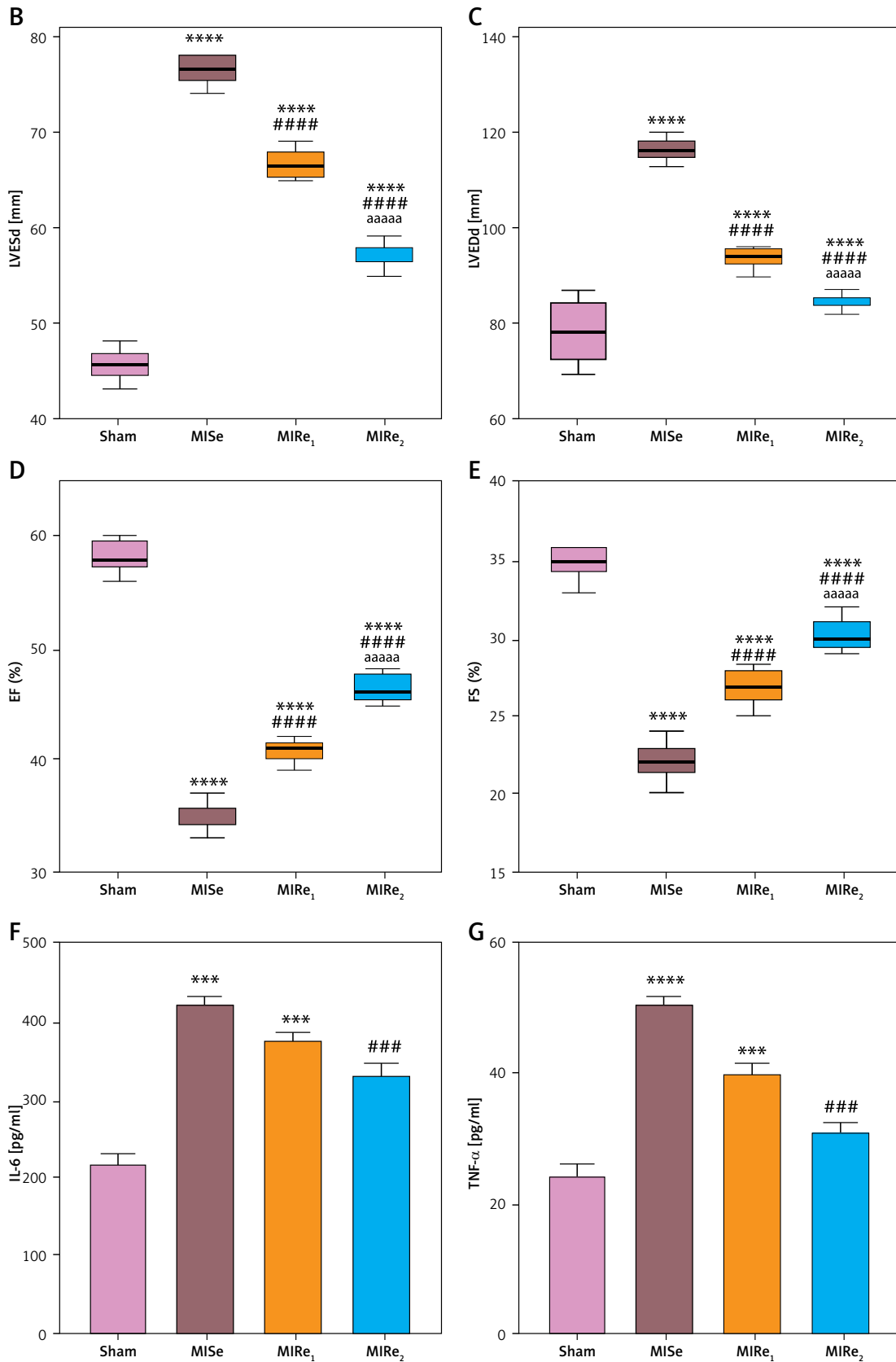


Figure 2. Cont. Quantitative analysis of (B) LV end-systolic diameter (LVESd), (C) LV end-diastolic diameter (LVEDd), (D) ejection fraction (EF), and (E) fractional shortening (FS). Myocardial inflammatory markers are shown as (F) IL-6 and (G) TNF-α. In the figure labels, MISe/MI indicates MI-sedentary; MIRE₁/Ren-1 indicates Mlex1; and MIRE₂/Ren-2 indicates Mlex3. * $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs. Sham; # $p < 0.05$, ### $p < 0.001$, #### $p < 0.0001$ vs. MI-sedentary

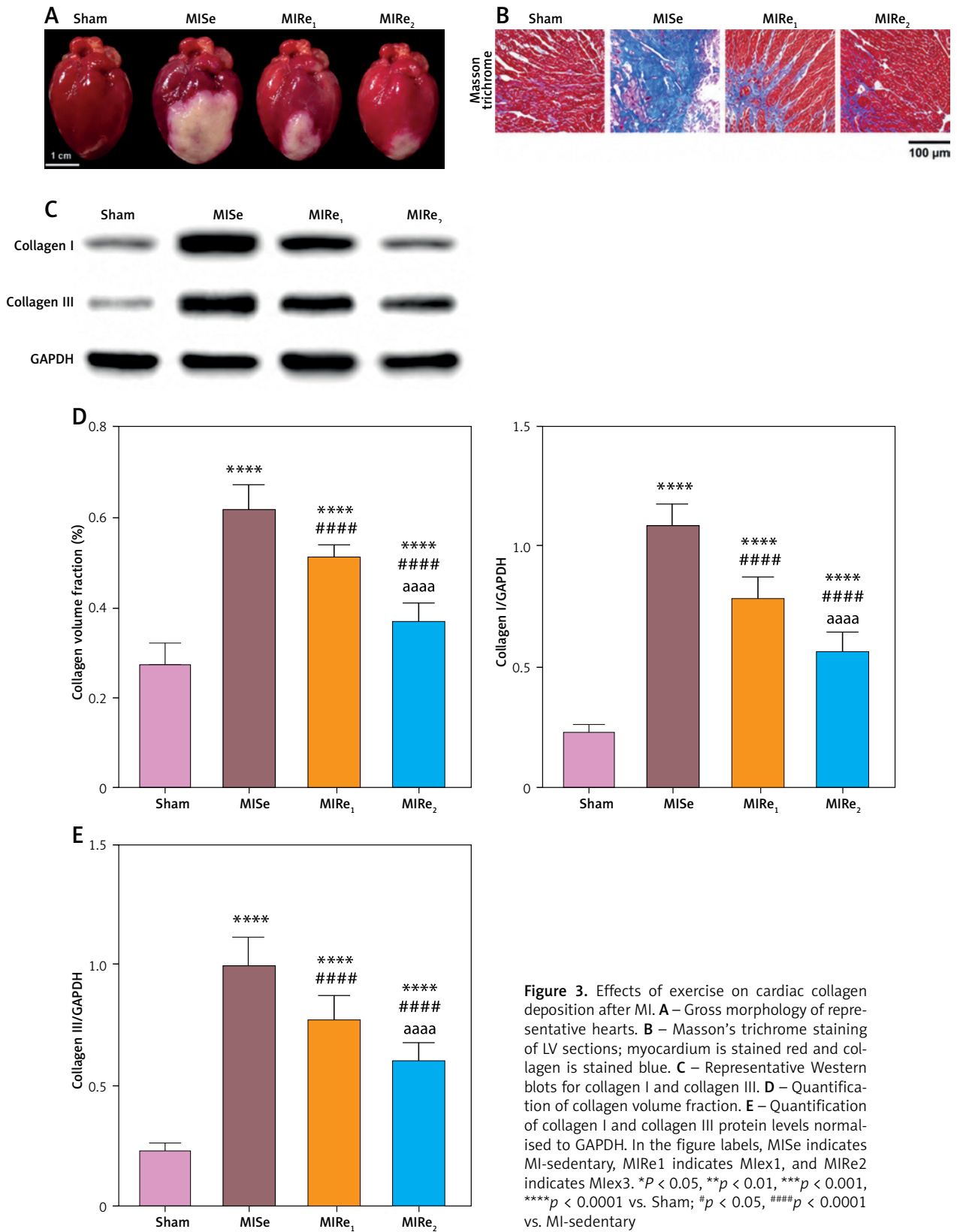


Figure 3. Effects of exercise on cardiac collagen deposition after MI. **A** – Gross morphology of representative hearts. **B** – Masson's trichrome staining of LV sections; myocardium is stained red and collagen is stained blue. **C** – Representative Western blots for collagen I and collagen III. **D** – Quantification of collagen volume fraction. **E** – Quantification of collagen I and collagen III protein levels normalised to GAPDH. In the figure labels, MISE indicates MI-sedentary, MIRE₁ indicates Mlex₁, and MIRE₂ indicates Mlex₃. * $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs. Sham; # $p < 0.05$, ##### $p < 0.0001$ vs. MI-sedentary

packed and highly organised, forming a well-organised 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 3 D). In both Mlex1 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 fibres. In all groups, by 12 weeks, no haemorrhage 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 Mlex1 following 4 weeks of exercise and in Mlex3 after 12 weeks of exercise (Figures 3 C, E).

Compared with the sham group, MI rats had a significantly greater amount of Ang II in their LV (Table I). Mlex3 showed a significant reduction in Ang II after 12 weeks of ET in the post-infarct LV; however, Mlex1 showed a lower Ang II level after 4 weeks of ET, but this difference was not significant.

Effects of exercise on AT1R/TGF- β 1 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 (Figures 4 A, 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- β 1 during post-infarct cardiac fibrosis, we next assessed TGF- β 1. TGF- β 1 protein expression was markedly increased in LV tissues of MI-sedentary rats and was attenuated by ET, particularly in the Mlex3 group (Figures 4 B, D).

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 (Figures 4 E–H). The reduction was more pronounced in the Mlex3 group than in the Mlex1 group. These data suggest that ET is associated with lower activity of the TGF- β 1/SMAD pathway in MI-induced cardiac fibrosis.

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- β 1, and phosphorylated SMAD2/3 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- β 1/SMAD signalling 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 ventricu-

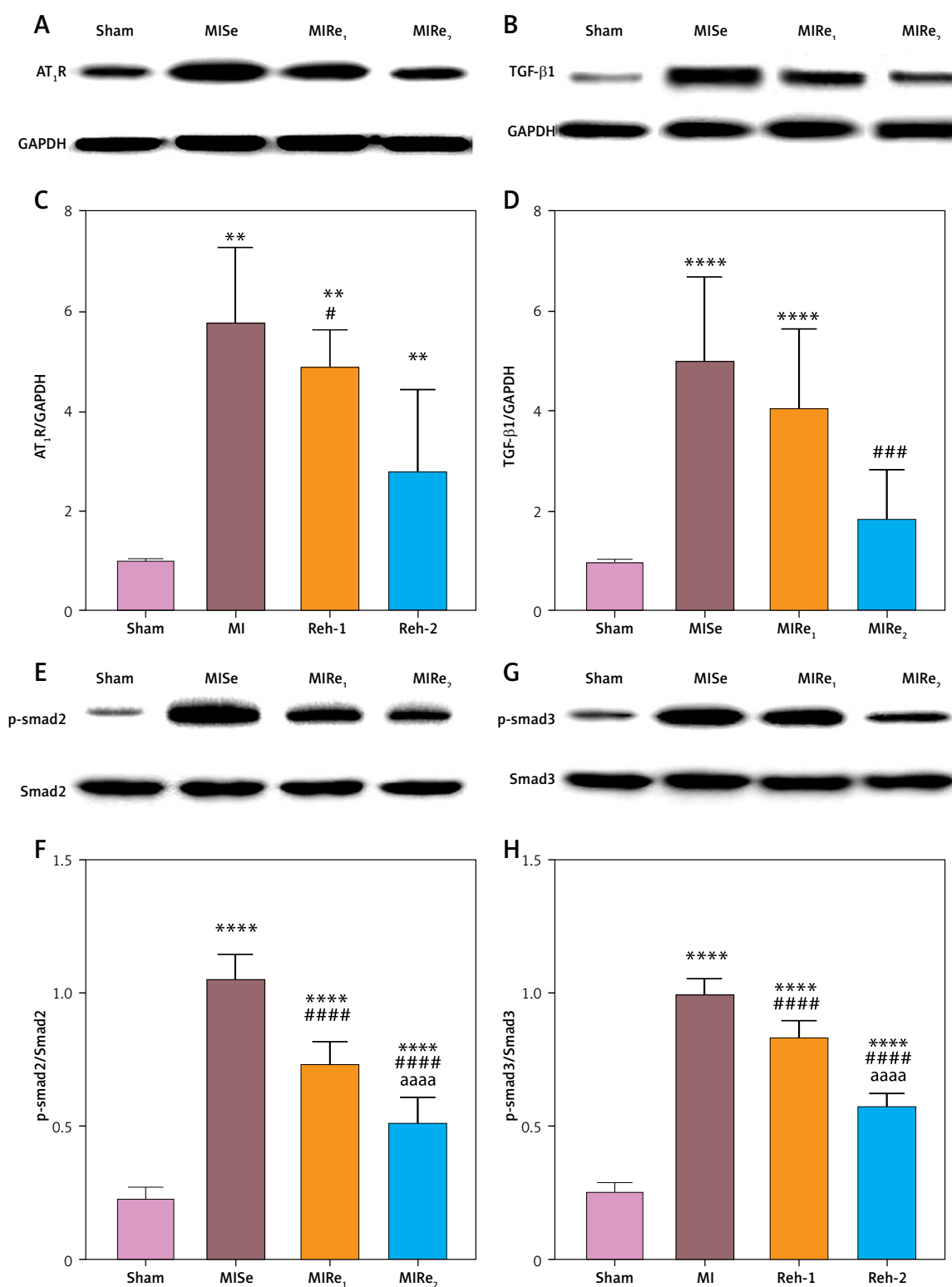


Figure 4. Effects of exercise on AT1R/TGF- β 1/SMAD signalling after MI. Representative Western blots for (A) AT1R and (B) TGF- β 1, with quantitative analysis of (C) AT1R/GAPDH and (D) TGF- β 1/GAPDH. Representative blots and quantification are also shown for (E, F) p-SMAD2/SMAD2 and (G, H) p-SMAD3/SMAD3. In the figure labels, MISe/MI indicates MI-sedentary; MIRe₁/Reh-1 indicates Mlex1; and MIRe₂/Reh-2 indicates Mlex3. * P < 0.05, ** p < 0.01, **** p < 0.0001 vs. Sham; # p < 0.05, ### p < 0.001, #### p < 0.0001 vs. MI-sedentary

lar remodeling, and prolongs survival in patients with MI and heart failure [34, 35]. Antagonism of AT₁R 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 signalling 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/AT₁R-mediated activation of TGF- β ₁/SMAD signalling 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 signalling in exercise-mediated effects. We previously confirmed that the AT₁R-specific degradation of SMAD7 decreased the inhibitory feedback regulation of TGF- β ₁/SMAD3/SMAD4 signalling, thereby promoting collagen accumulation in atrial fibrosis and remodeling [10, 11]. It is also reported that decreased SMAD7 expression, by activating TGF- β ₁/SMAD2/SMAD3 signalling, 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- β ₁/SMAD signalling [41, 42]. Similarly, exercise increases endothelial NO synthase (eNOS) activity and NO bioavailability [43]. NO can inhibit TGF- β ₁-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- β ₁/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- β ₁/SMAD signalling 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- β ₁ 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- β ₁/SMAD signalling. Direct pathway interventions are still needed to establish causality.

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- β inhibitor, or a SMAD modulator) must be performed to test causality of the observed correlation. Second, only one exercise intensity was 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 signalling 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.

In conclusion, this research provides evidence for the cardioprotective effect of ET on maintaining cardiac systolic function and reducing myocardial fibrosis following MI. The decrease in the signalling pathway Ang II/AT₁R/TGF- β ₁/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- β inhibition, or SMAD modulation) will be needed to establish a cause-and-effect relationship.

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Ethical 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 15 August 2020, under the title 'Mechanisms of Exercise-Induced Cardioprotection After Myocardial Infarction: Role of the AT1R/TGF- β 1/Smad Pathway'.

Conflict of interest

The authors declare no conflict of interest.

References

- Gerber Y, Weston SA, Enriquez-Sarano M, et al. Mortality associated with heart failure after myocardial infarction. *Circ Hear Fail* 2016; 9: e002460.
- Bulluck H, Dharmakumar R, Arai AE, Berry C, Hausenloy DJ. Cardiovascular magnetic resonance in acute ST-segment-elevation myocardial infarction: recent advances, controversies, and future directions. *Circulation* 2018; 137: 1949-64.
- Prabhu SD, Frangogiannis NG, Service M, Einstein A. The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis. *Circ Res* 2017; 119: 91-112.
- Talman V, Ruskoaho H. Cardiac fibrosis in myocardial infarction – from repair and remodeling to regeneration. *Cell Tissue Res* 2016; 365: 563-81.
- Frangogiannis NG. Pathophysiology of myocardial infarction. *Compr Physiol* 2015; 5: 1841-75.
- Aras O, Messina SA, Shirani J, Eckelman WC, Dilsizian V. The role and regulation of cardiac angiotensin-converting enzyme for noninvasive molecular imaging in heart failure. *Curr Cardiol Rep* 2007; 9: 150-8.
- Rosenkranz S. TGF- β 1 and angiotensin networking in cardiac remodeling. *Cardiovasc Res* 2004; 63: 423-32.
- Meng XM, Tang PMK, Li J, Lan HY. TGF- β /Smad signaling in renal fibrosis. *Front Physiol* 2015; 6: 82.
- Miyazawa K, Miyazono K. Regulation of TGF- β family signaling by inhibitory smads. *Cold Spring Harb Perspect Biol* 2017; 9: a022095.
- He X, Zhang K, Gao X, et al. Rapid atrial pacing induces myocardial fibrosis by down-regulating Smad7 via microRNA-21 in rabbit. *Heart Vessels* 2016; 31: 1696-708.
- He X, Gao X, Peng L, et al. Atrial fibrillation induces myocardial fibrosis through angiotensin II type 1 receptor-specific Arkadia-mediated downregulation of Smad7. *Circ Res* 2011; 108: 164-75.
- Zhang K, He X, Zhou Y, et al. Atorvastatin ameliorates radiation-induced cardiac fibrosis in rats. *Radiat Res* 2015; 184: 611-20.
- Giallauria F, Acampa W, Ricci F, et al. Effects of exercise training started within 2 weeks after acute myocardial infarction on myocardial perfusion and left ventricular function: a gated SPECT imaging study. *Eur J Prev Cardiol* 2012; 19: 1410-9.
- Tucker WJ, Beaudry RI, Liang Y, et al. Meta-analysis of exercise training on left ventricular ejection fraction in heart failure with reduced ejection fraction: a 10-year update. *Prog Cardiovasc Dis* 2019; 62: 163-71.
- Lawler PR, Filion KB, Eisenberg MJ. Efficacy of exercise-based cardiac rehabilitation post-myocardial infarction: a systematic review and meta-analysis of randomized controlled trials. *Am Heart J* 2011; 162: 571-84.e2.
- Zucker IH, Schultz HD, Patel KP, Wang H. Modulation of angiotensin II signaling following exercise training in heart failure. *Am J Physiol Hear Circ Physiol* 2015; 308: H781-91.
- Wan W, Powers AS, Li J, Ji L, Erikson JM, Zhang JQ. Effect of post-myocardial infarction exercise training on the renin-angiotensin-aldosterone system and cardiac function. *Am J Med Sci* 2007; 334: 265-73.
- Puhl SL, Müller A, Wagner M, et al. Exercise attenuates inflammation and limits scar thinning after myocardial infarction in mice. *Am J Physiol Hear Circ Physiol* 2015; 309: H345-59.
- Jia D, Hou L, Lv Y, Xi L, Tian Z. Postinfarction exercise training alleviates cardiac dysfunction and adverse remodeling via mitochondrial biogenesis and SIRT1/PGC-1 α /PI3K/Akt signaling. *J Cell Physiol* 2019; 234: 23705-18.
- Kraljevic J, Marinovic J, Pravdic D, et al. Aerobic interval training attenuates remodelling and mitochondrial dysfunction in the post-infarction failing rat heart. *Cardiovasc Res* 2013; 99: 55-64.
- Kröpfl JM, Spengler CM, Frobert A, Ajalbert G, Giraud MN. Myocardial infarction does not affect circulating haematopoietic stem and progenitor cell self-renewal ability in a rat model. *Exp Physiol* 2018; 103: 1-8.
- Xu X, Wan W, Ji L, et al. Exercise training combined with angiotensin II receptor blockade limits post-infarct ventricular remodelling in rats. *Cardiovasc Res* 2008; 78: 523-32.
- Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* 1978; 58: 1072-83.
- Rennison JH, McElfresh TA, Okere IC, et al. High-fat diet postinfarction enhances mitochondrial function and does not exacerbate left ventricular dysfunction. *Am J Physiol Hear Circ Physiol* 2007; 292: 1498-506.
- Luo J, Gao X, Peng L, Sun H, Dai G. Effects of hydrochlorothiazide on cardiac remodeling in a rat model of myocardial infarction-induced congestive heart failure. *Eur J Pharmacol* 2011; 667: 314-21.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods* 2001; 25: 402-8.
- Anderson L, Oldridge N, Thompson DR, et al. Exercise-based cardiac rehabilitation for coronary heart disease cochrane systematic review and meta-analysis. *J Am Coll Cardiol* 2016; 67: 1-12.
- Belardinelli R, Georgiou D, Cianci G, Purcaro A. 10-year exercise training in chronic heart failure: a randomized controlled trial. *J Am Coll Cardiol* 2012; 60: 1521-8.
- Wang Y, Tian M, Mi C, et al. Exercise protects the heart against myocardial infarction through upregulation of miR-1192. *Biochem Biophys Res Commun* 2020; 521: 1061-9.
- Guizoni DM, Oliveira-Junior SA, Noor SLR, et al. Effects of late exercise on cardiac remodeling and myocardial

- calcium handling proteins in rats with moderate and large size myocardial infarction. *Int J Cardiol* 2016; 221: 406-12.
31. Marijianowski MMH, Teeling P, Becker AE. Remodeling after myocardial infarction in humans is not associated with interstitial fibrosis of noninfarcted myocardium. *J Am Coll Cardiol* 1997; 30: 76-82.
 32. Van Aelst LNL, Voss S, Carai P, et al. Osteoglycin prevents cardiac dilatation and dysfunction after myocardial infarction through infarct collagen strengthening. *Circ Res* 2015; 116: 425-36.
 33. Yengo CM1, Zimmerman SD, McCormick RJ TD. Exercise training post-MI favorably modifies heart extracellular matrix in the rat. *Med Sci Sport* 2012; 44: 1005-12.
 34. Solomon SD, Skali H, Anavekar NS, et al. Changes in ventricular size and function in patients treated with valsartan, captopril, or both after myocardial infarction. *Circulation* 2005; 111: 3411-9.
 35. Granger CB, McMurray JJV, Yusuf S, et al. CHARM-Alternative trial (ARB CHF). *Lancet* 2003; 362: 772-6.
 36. Lim BK, Park J, Park SJ, et al. Fimasartan for remodeling after myocardial infarction. *J Clin Med* 2019; 8: 366.
 37. Huang BS, Chen A, Ahmad M, Wang HW, Leenen FHH. Mineralocorticoid and AT1 receptors in the paraventricular nucleus contribute to sympathetic hyperactivity and cardiac dysfunction in rats post myocardial infarct. *J Physiol* 2014; 592: 3273-86.
 38. Haack KKV, Engler CW, Papoutsis E, Pipinos II, Patel KP, Zucker IH. Parallel changes in neuronal at1r and grk5 expression following exercise training in heart failure. *Hypertension* 2012; 60: 354-61.
 39. Lee Y, Kwak HB, Hord J, Kim JH, Lawler JM. Exercise training attenuates age-dependent elevation of angiotensin II type 1 receptor and Nox2 signaling in the rat heart. *Exp Gerontol* 2015; 70: 163-73.
 40. Liang ZG, Yao H, Xie RS, Gong CL, Tian Y. MicroRNA-20b-5p promotes ventricular remodeling by targeting the TGF- β /Smad signaling pathway in a rat model of ischemia-reperfusion injury. *Int J Mol Med* 2018; 42: 975-87.
 41. Hang PZ. 7,8-Dihydroxyflavone alleviates cardiac fibrosis by restoring circadian signals via downregulating Bmal1/Akt pathway. *Eur J Pharmacol* 2023; 938: 175405.
 42. Zhu Y, Ran Y, Fu T, et al. Cardiomyocyte-derived BDNF restricts cardiac fibrosis by decreasing the activity of the TGF- β /Smad2/3 pathway and increasing Smad7 expression. *Front Cell Dev Biol* 2026; 14: 1786720.
 43. Paravlic AH, Drole K. Effects of aerobic training on brachial artery flow-mediated dilation in healthy adults: a meta-analysis of inter-individual response differences in randomized controlled trials. *BMC Sports Sci Med Rehabil* 2025; 17: 72.
 44. Andrews KL, Triggie CR, Ellis A. NO and the vasculature: where does it come from and what does it do? *Heart Fail Rev* 2002; 7: 423-45.
 45. Heger J, Warga B, Meyering B, et al. TGF β receptor activation enhances cardiac apoptosis via SMAD activation and concomitant NO release. *J Cell Physiol* 2011; 226: 2683-90.