To appear in Exercise Physiology and Performance (EPP)
Received: 2025-08-04 Revised: 2025-9-13 Accepted: 2025-10-16
DOI: https://doi.org/10.71951/epp.2025.202508041214016

Effects of Continuous and Interval Aerobic Training on the Expression of TGF-β and Type I and III Collagen Genes (Fibrotic Markers) in the Myocardium of Rats with Myocardial Infarction

Farimah Kazemi Bani[1] , Jamshid Banaei Borogeni[1*], Elham Eftekhari[1] , Hamid Zahedi[1]

1. Department of Physical Education, Na.C., Islamic Azad University, Najafabad, Iran.

Abstract

Background: Myocardial infarction (MI), a leading cause of death worldwide, often leads to myocardial fibrosis due to excess type I and III collagen and TGF-β pathway activation, impairing heart function. This study aimed to investigate the effects of moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) on the expression of TGF-β, type I and III collagen genes in a rat model of myocardial infarction, to explore non-invasive strategies for reducing cardiac fibrosis.

Methods: In this laboratory trial, 40 male Wistar rats were divided into four groups: healthy control (Ct), myocardial infarction without training (MI), MI with moderate-intensity continuous training (MIMCT), and MI with high-intensity interval training (MIHIIT). Myocardial infarction was induced by ligation of the coronary artery. Training protocols were performed for 8 weeks (5 days/week). MICT was conducted at 60%-75% VO2max for 45 minutes per session. HIIT consisted of seven sets of high- and low-intensity intervals: 4 minutes at 80%-90% VO2max and 3 minutes at 60% VO2max. Gene expression was measured using Real-Time PCR. Data were analyzed using SPSS, with two-way ANOVA for main effects, Tukey post hoc for pairwise comparisons, and independent t-tests to compare training groups ($p < 0.05$).

Results: TGF-β expression increased significantly in the MI group compared to Ct ($P < 0.001$), but decreased significantly in both training groups (MIHIIT: 50%; MIMCT: 30%; $P < 0.001$). The difference between MIHIIT and MIMCT was significant ($P = 0.012$). Expression of type I collagen (MIHIIT: 40%; MIMCT: 37%) and type III collagen (MIHIIT: 44%; MIMCT: 40%) also decreased, but differences between training types were not significant ($P > 0.05$).

Conclusion: Aerobic training, especially HIIT, significantly reduces TGF-β expression and may attenuate cardiac fibrosis and heart failure risk. HIIT is recommended as part of cardiac rehabilitation for MI patients.

Keywords: Myocardial infarction, cardiac fibrosis, TGF-β, aerobic exercise, collagen

* **Corresponding author:** banaii9557@iau.ac.ir

Introduction

Myocardial infarction (MI), or heart attack, remains one of the leading causes of mortality worldwide, accounting for approximately 17.9 million cardiovascular-related deaths annually (1). MI occurs when coronary arteries become occluded, leading to ischemia and necrosis of cardiac tissue. This event triggers pathological remodeling, including inflammation and fibrosis, which impairs cardiac function and increases the risk of heart failure (2). Fibrosis, characterized by the excessive deposition of extracellular matrix (ECM)—particularly type I and III collagens—stiffens the myocardium and disrupts its contractility (3). Given the significant burden of cardiovascular diseases on global public health systems, identifying strategies to mitigate fibrotic remodeling post-MI is imperative. Exercise training, as a key component of cardiac rehabilitation, offers a non-invasive and promising approach to improving outcomes; however, its specific impact on fibrosis requires further investigation.

Fibrotic remodeling following MI is governed by complex molecular pathways, among which transforming growth factor-beta (TGF-β) plays a central role. TGF-β activates fibroblasts, resulting in the synthesis of type I and III collagens—primary components of the ECM (4). Type I collagen provides tensile strength, while type III collagen enhances elasticity; disproportionate expression of these collagens after MI can lead to the formation of stiff, inefficient scars (5). The TGF-β/Smad signaling pathway—especially via Smad3—directly regulates collagen gene expression (4). Moreover, studies have shown that aerobic exercise modulates this pathway, likely through its anti-inflammatory and antioxidant effects, thereby reducing fibrosis (6). However, the specific influence of different aerobic training modalities (interval vs. continuous) on these mechanisms remains poorly understood and necessitates targeted research.

Optimizing exercise as a therapeutic tool for modulating fibrosis after MI is challenged by several barriers. Variability in training protocols (e.g., intensity, duration) across studies makes it difficult to draw standardized conclusions (7). While continuous aerobic training (CAT), with its potential for delivering unique physiological benefits, has been extensively investigated, the effects of interval aerobic training (IAT) are less explored (8).

Additionally, the precise molecular mechanisms linking exercise to alterations in fibrotic markers, particularly in the context of MI, remain inadequately understood. Previous research has emphasized the potential of physical activity to reduce cardiac fibrosis post-MI. Animal studies have demonstrated that continuous aerobic training can decrease TGF-β gene expression and collagen deposition (9). Huang et al. (2024) reported that treadmill exercise in mice inhibited the p300/CBP-associated factor, a TGF-β co-activator, thereby attenuating remodeling (10). Similarly, Peyronnel et al. (2024) observed reduced fibrosis in exercised rats under inflammatory conditions (9,10). In humans, high-intensity interval training (HIIT) has shown superior effects over moderate-intensity continuous training (MICT) in reversing ventricular remodeling in heart failure (11), although its effects on fibrotic markers remain less well documented. While these studies provide valuable insights, they lack direct comparisons between continuous and interval training in relation to fibrotic markers.

Based on the available evidence, significant gaps remain in understanding the role of exercise on myocardial fibrotic indices. Findings suggest that the effects of continuous aerobic training on type I and III collagen are inconsistent; some studies report reductions in both (12), while others indicate a selective decrease in type I collagen, preserving elasticity (6). Conversely, the impact of interval aerobic training on these markers has been less thoroughly investigated. Mehdipoor et al. (2021) noted that interval aerobic training modulates myocardial fibrosis in infarcted rats, but TGF-β and collagen levels were not assessed in that study (13). Thus, whether continuous and interval aerobic training differentially affect TGF-β and collagen expression remains to be systematically examined to resolve discrepancies and fill knowledge gaps. The present study introduces a novel comparison of the effects of continuous and interval aerobic training on the gene expression of TGF-β and type I and III collagens in a post-MI rat model. This represents a distinct approach from previous research, which primarily focused on continuous protocols. Such work is essential to address identified gaps—particularly inconsistent collagen findings and the uncharted potential of interval aerobic training—and to ensure that exercise prescriptions for cardiac rehabilitation are optimized. These insights are critical for advancing targeted therapeutic strategies against myocardial fibrosis.

Material and Methods

This study was conducted as a laboratory-based clinical trial. From a methodological and objective perspective, it is considered an applied research project aimed at expanding knowledge in the field of non-pharmacological strategies for improving heart diseases, particularly after myocardial infarction (MI). In this study, 40 aged male Wistar rats (10–12 weeks old, mean weight 300 ± 50 g) were used. The animals were obtained from the Laboratory Animal Breeding and Reproduction Center in Marvdasht (Marvdasht, Iran). The rats were housed in transparent polycarbonate cages (four rats per cage) under controlled environmental conditions: a 12 h light/12 h dark cycle, temperature of 22 ± 2 °C, and relative humidity of 50 ± 5%. Food (standard rat chow) and water were available ad libitum. All laboratory procedures were approved by the institutional ethics committee and conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and ARRIVE guidelines. Following the induction of myocardial infarction in 30 rats, the animals were randomly assigned to one of four groups:

1. Healthy control group (Ct): healthy rats without any intervention.
2. MI group without exercise (MI): rats with myocardial infarction that did not undergo training.
3. MI + moderate-intensity continuous training group (MIMCT): MI rats subjected to moderate-intensity continuous aerobic training.
4. MI + high-intensity interval training group (MIHIIT): MI rats subjected to high-intensity interval aerobic training.

The sample size (10 rats per group) was determined based on statistical calculations and previous standards to ensure adequate statistical power for analysis.

The myocardial infarction model was induced by ligation of the left anterior descending (LAD) coronary artery. Rats were anesthetized using a combination of ketamine (50 mg/kg) and xylazine (10 mg/kg). After fixation on the surgical table, a thoracotomy was performed through a 10 mm incision between the third and fourth intercostal spaces. The LAD artery was ligated 1–2 mm below its origin using a 6-0 polypropylene suture. Successful ligation was confirmed by myocardial discoloration (pallor) and ST-segment elevation on the electrocardiogram. The chest

was then closed in three layers using 5-0 prolene sutures. Forty-eight hours post-surgery, echocardiography was performed using a 10 MHz probe to confirm MI based on ejection fraction and fractional shortening. To reduce pain and prevent infection, tramadol (10 mg/kg) and cefazolin (20 mg/kg) were administered for three days following surgery.

After one week of recovery, the training groups (continuous and interval) were familiarized with treadmill running for two weeks (three sessions per week, 10–15 minutes per session at 10–15 m/min). Then, maximal aerobic capacity was assessed using an incremental treadmill test (starting at 6 m/min with 2 m/min increases every 2 minutes until exhaustion) as described by Barcelos et al. (2017) (14). Training protocols were performed over 8 weeks, five days per week, at 0° incline. These protocols were adapted from Nori et al. (2023). The continuous training group exercised progressively at an intensity of 60% to 75% of VO_2max for 45 minutes per session. The interval training group completed 7 sets of alternating high- and low-intensity bouts: high-intensity bouts at 80%–90% of VO_2max for 4 minutes, and low-intensity bouts at 60% of VO_2max for 3 minutes (15).

Seventy-two hours after the final training session, rats were anesthetized with CO_2 gas. Myocardial tissue was collected from the infarcted region, rinsed in saline, and stored at −80°C. Total RNA was extracted from myocardial samples using TRIzol reagent (Invitrogen, USA) and treated with DNase I to remove genomic DNA contamination. RNA quality and purity were assessed by measuring the 260/280 nm absorbance ratio using a NanoDrop spectrophotometer (Thermo Fisher Scientific); ratios above 1.8 were considered acceptable for purity. One microgram of RNA was reverse-transcribed into cDNA using the Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific). Gene expression levels of TGF-β, collagen type I, and collagen type III were quantified by real-time PCR using an ABI Prism 7500 system. Target-specific primers were designed by SinaColon (Iran). The thermal cycling program consisted of an initial denaturation at 95°C for 15 minutes, followed by 40 cycles of denaturation (95°C for 30 s), annealing (60°C for 30 s), and extension (72°C for 30 s). A melting curve analysis was conducted between 55°C and 95°C. Gene expression was analyzed using the comparative ΔΔCt method, with GAPDH as the housekeeping gene.

Data were analyzed using SPSS version 26. Two-way ANOVA was employed to assess the effects of exercise type (MICT vs. HIIT) and disease status (healthy control vs. MI) on the gene expression levels of TGF-β, collagen type I, and type III. If a significant effect was observed, Tukey's post-hoc test was used for pairwise comparisons. Additionally, independent t-tests were conducted to compare MICT and HIIT groups for each gene. A significance level of $p < 0.05$ was considered for all analyses. All procedures were conducted in accordance with the Declaration of Helsinki and were approved by the institutional ethics committee (Ethics Code: IR.xxx.REC.xxx).

Results

This section presents the findings on the effects of moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) on the gene expression of TGF-β, collagen type I, and collagen type III in the myocardial tissue of rats with induced myocardial infarction (MI). The experimental groups included: healthy control (Ct), myocardial infarction without training (MI), myocardial infarction with moderate-intensity continuous training (MIMCT), and myocardial infarction with high-intensity interval training (MIHIIT). Gene expression was measured using Real-Time PCR, and protein levels were assessed by ELISA. Each gene is analyzed separately, and the results are presented using tables and bar graphs.

To evaluate the effects of continuous and interval aerobic training on TGF-β gene expression, a two-way ANOVA was conducted to assess the influence of group and training type. The results are summarized in Table 1, titled "Mean TGF-β Gene Expression in Experimental Groups.

Table 1. Mean TGF-β Gene Expression in Experimental Groups Post-MI and Training Interventions

Group	Relative Expression (Mean ± SD)	*P* Value (vs. MI group)
Ct	1.0 ± 0.2	< 0.001
MI	3.0 ± 0.5	-
MIMCT	2.1 ± 0.4	0.003

MIHIIT	1.5 ± 0.3	< 0.001

As shown in Table 1, TGF-β gene expression significantly increased in the MI group compared to the healthy control (Ct) group ($P < 0.001$). Both continuous and interval training significantly reduced TGF-β expression compared to the MI group (MIMCT: $P = 0.003$; MIHIIT: $P < 0.001$). However, the MIHIIT group demonstrated a greater reduction (50% compared to MI) than the MIMCT group (30% compared to MI). To further assess the difference between training types, an independent *t*-test was conducted, which revealed a significant difference between MIMCT and MIHIIT ($P = 0.012$). This test is suitable for comparing the direct impact of two training types by allowing pairwise analysis of means.

To examine the effects of aerobic training on collagen type I gene expression, a two-way ANOVA was conducted to determine group differences and the impact of training. The findings are shown in Table 2, titled "Mean Collagen Type I Gene Expression in Experimental Groups.

Table 2. Mean Collagen Type I Gene Expression in Experimental Groups Post-MI and Training Interventions

Group	Relative Expression (Mean ± SD)	*P* Value (vs. MI group)
Ct	1.0 ± 0.2	< 0.001
MI	3.5 ± 0.6	-
MIMCT	2.2 ± 0.5	0.002
MIHIIT	2.1 ± 0.4	0.001

As seen in Table 2, collagen type I expression was significantly elevated in the MI group compared to the Ct group ($P < 0.001$). Both MICT and HIIT significantly reduced this expression (MIMCT: $P = 0.002$; MIHIIT: $P = 0.001$). The reduction was approximately 37% in MIMCT and 40% in MIHIIT compared to MI. An independent *t*-test showed that the difference between MIMCT and MIHIIT was not statistically significant ($P = 0.45$). This test enables direct comparison of the two exercise protocols in different groups by evaluating pairwise differences.

To assess the effects of aerobic training on collagen type III gene expression, a two-way ANOVA was performed to examine group differences and training effects. The results are presented in Table 3, titled "Mean Collagen Type III Gene Expression in Experimental Groups.

Table 3. Mean Collagen Type III Gene Expression in Experimental Groups Post-MI and Training Interventions

Group	Relative Expression (Mean ± SD)	*P* Value (vs. MI group)
Ct	1.0 ± 0.2	< 0.001
MI	3.2 ± 0.6	-
MIMCT	1.9 ± 0.4	0.001
MIHIIT	1.8 ± 0.3	< 0.001

As shown in Table 3, collagen type III gene expression was significantly higher in the MI group compared to the Ct group ($P < 0.001$). Both training interventions significantly reduced expression levels (MIMCT: $P = 0.001$; MIHIIT: $P < 0.001$). The reduction was approximately 40% in MIMCT and 44% in MIHIIT compared to MI. An independent *t*-test showed no significant difference between the two training groups ($P = 0.38$), indicating similar effects of both training modalities on collagen type III expression. To evaluate the overall effects of MICT and HIIT on genes associated with fibrotic remodeling in damaged myocardium, the relative expression of the three key genes—TGF-β, collagen type I, and collagen type III—was examined across the four study groups. Figure 1 presents the combined expression data as a grouped bar chart. These data were derived from Real-Time PCR and statistically evaluated using ANOVA and *t*-tests. Mean values and standard deviations, along with standard errors, are displayed for each gene in all groups.

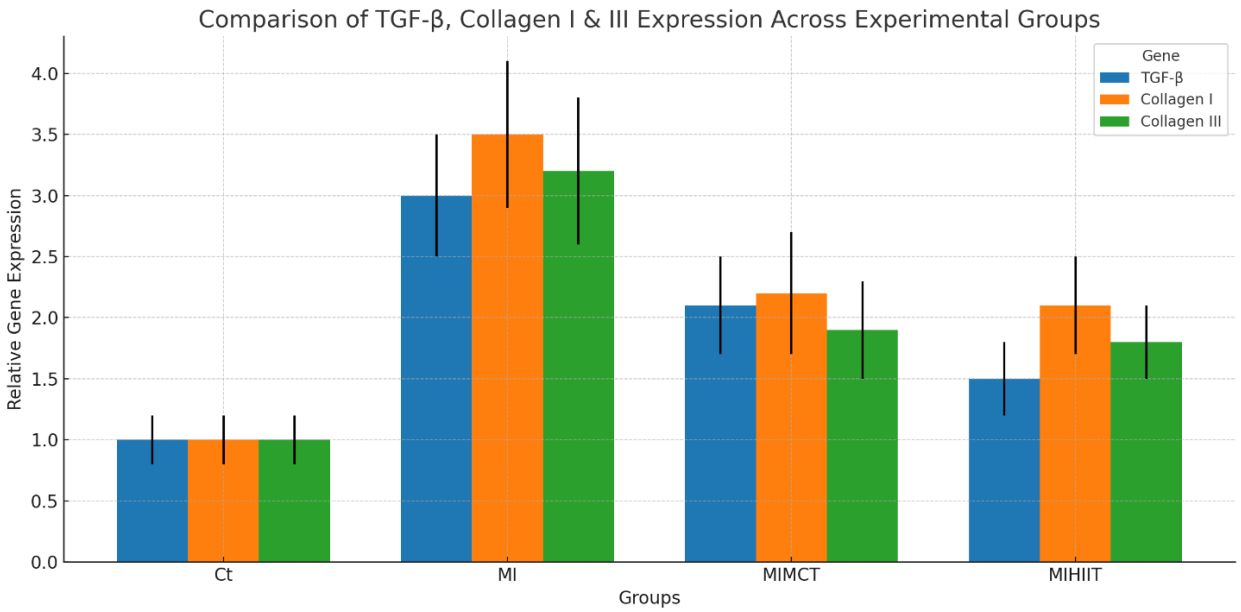

Figure 1. Comparative Expression of TGF-β, Collagen Type I, and Collagen Type III Across Experimental Groups

According to the data presented in Figure 1, myocardial infarction led to a significant increase in the expression of all three fibrotic genes compared to the healthy control group. Exercise training, particularly interval aerobic training, markedly reduced TGF-β expression: MIHIIT (1.5 units) vs. MI (3.0 units), $P < 0.001$. Continuous training also reduced TGF-β expression to 2.1 units ($P = 0.003$), but to a lesser extent. A similar pattern was observed for collagen types I and III. Both exercise modalities significantly reduced gene expression compared to MI, but the differences between MICT and HIIT for collagen genes were not statistically significant. These results suggest that interval training exerts a stronger suppressive effect on TGF-β, a key regulator of fibrotic signaling, while both training modalities similarly affect collagen gene expression. This highlights the importance of exercise type and intensity in cardiac tissue remodeling post-MI and supports the potential of interval training as a more effective intervention in reducing fibrotic responses.

To more precisely compare the effects of MICT and HIIT on the expression of TGF-β, collagen type I, and collagen type III, independent *t*-tests were conducted. These tests compared the MIMCT and MIHIIT groups pairwise for each gene. The results are shown in Table 4, titled Comparison of Exercise Types on Gene Expression.

Table 4. Comparison of the Effects of MICT and HIIT on Gene Expression (MIMCT vs. MIHIIT)

Gene	Group	Relative Expression (Mean ± SD)	*P* Value
TGF-β	MIMCT	2.1 ± 0.4	0.012
	MIHIIT	1.5 ± 0.3	
Collagen Type I	MIMCT	2.2 ± 0.5	0.45
	MIHIIT	2.1 ± 0.4	
Collagen Type III	MIMCT	1.9 ± 0.4	0.38
	MIHIIT	1.8 ± 0.3	

As shown in Table 4, HIIT significantly reduced TGF-β gene expression compared to MICT (P = 0.012), confirming that interval training has a stronger inhibitory effect on this gene. However, no significant differences were observed between the two exercise modalities for collagen type I (P = 0.45) and collagen type III (P = 0.38). These results suggest that while both exercise types exert similar effects on collagen gene expression, interval training may be a more effective strategy for modulating TGF-β, a central factor in fibrotic remodeling.

Discussion

The present study focused on evaluating the effects of two aerobic exercise protocols—Moderate-Intensity Continuous Training (MICT) and High-Intensity Interval Training (HIIT)—on the expression of myocardial fibrosis-related genes, particularly TGF-β, type I collagen, and type III collagen, in a rat model of myocardial infarction (MI). The results demonstrated that both exercise types significantly reduced the expression of these genes compared to the MI control group. However, HIIT showed a marked reduction (up to 50%) in TGF-β gene expression versus the MI group, reflecting a significant superiority over the 30% reduction achieved by MICT (P=0.012). In contrast, for type I and type III collagen genes, both protocols exhibited similar effects with no statistically significant difference between MICT and HIIT (P>0.05). These findings partially align with the study's initial hypothesis, which predicted greater fibrosis reduction with HIIT due to its higher intensity and antifibrotic signaling potential, underscoring the critical role of exercise intensity in modulating TGF-β expression.

This study revealed that both MICT and HIIT significantly reduced TGF-β gene expression in rats with cardiac infarction. These results align with prior research: Huang et al. (2024) reported decreased TGF-β expression following sustained aerobic exercise, and Liu et al. (2023) demonstrated that aerobic training modulates the TGF-β pathway via anti-inflammatory and antioxidant effects (10). Zhang et al. (2025) further showed that aerobic exercise reduces hepatic fibrosis by regulating the TGF-β/SMAD2 pathway, consistent with our cardiac findings (16). Wisloff et al. (2007) confirmed the superiority of HIIT in improving heart failure, while Wang et al. (2019) revealed that HIIT inhibits the TGF-β pathway by modulating miRNAs such as miR-29a (17). Zhao et al. (2025) also noted in their review that exercise reduces profibrotic factors like TGF-β, reinforcing our results (18).

The greater reduction in TGF-β in the HIIT group likely stems from its higher intensity, which may suppress TGF-β expression via anti-inflammatory/antioxidant pathways and miRNA regulation (e.g., miR-29a, miR-101a). Fu et al. (2024) demonstrated that exercise inhibits the TGF-β/Smad3 pathway by increasing miR-126 in endothelial progenitor cell-derived exosomes (19). Kawanishi et al. (2012) also reported reduced systemic inflammation with high-intensity exercise, indirectly influencing TGF-β. Given these findings, HIIT is proposed as a more effective intervention to reduce cardiac fibrosis in post-MI patients within cardiac rehabilitation programs (20). This approach may improve cardiac function and reduce heart failure risk, particularly in patients responsive to higher-intensity interventions.

In this study, both MICT and HIIT significantly reduced type I collagen expression (by 37% and 40%, respectively), but the intergroup difference was nonsignificant. These results align with Li et al. (2021) and Liu et al. (2023), who reported collagen reduction with exercise (12, 6). Heinemeier et al. (2003) observed increased type I collagen synthesis in human tendon tissue with exercise, contrasting with our reduction—possibly due to tissue differences (cardiac vs. tendon) (21). Aispuru-Lanche et al. (2023) reported greater potential for HIIT in cardiac regeneration, conflicting with our nonsignificant difference (8). Nan et al. (2019) showed that mechanical force increases type I collagen synthesis in gingival fibroblasts via TGF-β, highlighting tissue-specific exercise effects (22).

The reduction in type I collagen in both exercise groups may result from diminished myofibroblast activity and modulation of the TGF-β/Smad pathway. Jiao et al. (2025) confirmed this pathway's

key role in collagen regulation (4). Ma et al. (2025) also noted that exercise reduces organ fibrosis by modulating molecular pathways like TGF-β1/Smad (23). Given the comparable effects of MICT and HIIT, aerobic exercise is recommended as a general strategy to mitigate cardiac fibrosis. For patients with advanced fibrosis, adjusting exercise intensity/duration may optimize outcomes. Future research should explore protocols to further reduce type I collagen.

For type III collagen, both protocols significantly reduced gene expression (by 40% and 44%, respectively), with no significant intergroup difference. This aligns with Li et al. (2021), who reported collagen reduction with exercise (12). However, Xiong et al. (2023) observed no significant collagen changes in physiological cardiac hypertrophy, possibly due to model differences (disease vs. health) (24). Roozbayani et al. (2016) confirmed reduced cardiac fibrosis in diabetic rats with exercise, supporting our findings (25). The nonsignificant difference between MICT and HIIT resonates with Moghaddam et al. (2017), who reported minimal differences between exercise types in breast cancer (26).

The reduction in type III collagen likely arises from modulation of the TGF-β/Smad pathway and reduced myofibroblast activity. Ortega et al. (2024) emphasized the balance between type I and III collagen for myocardial elasticity (5). Wang et al. (2025) also demonstrated that exercise-induced mechanical mechanisms regulate extracellular matrix synthesis (27). Given the comparable efficacy of both protocols, aerobic exercise is proposed as a noninvasive intervention to preserve collagen balance and improve myocardial elasticity in cardiac fibrosis patients. Future studies should examine long-term effects on cardiac function to refine therapeutic strategies.

The significant reduction in TGF-β with HIIT may involve activation of intensity-dependent anti-inflammatory/antioxidant pathways. This process likely occurs via suppression of proinflammatory cytokines (e.g., IL-6, TNF-α), which downregulate TGF-β expression (20). Additionally, HIIT may exert effects through epigenetic regulation of miRNAs (e.g., miR-29a, miR-101a), previously identified as TGF-β pathway inhibitors (18). By targeting TGF-β-related mRNAs, these miRNAs reduce profibrotic protein production, thereby attenuating fibrosis. Such mechanisms indicate that higher-intensity HIIT not only modulates inflammation but also enhances antifibrotic effects via gene regulation.

The similar effects of HIIT and MICT on type I and III collagen levels likely reflect reduced myofibroblast activity and modulation of the TGF-β/Smad pathway, activated by both protocols (4). However, the nonsignificant intergroup difference may indicate insufficient exercise intensity/duration to differentiate collagen reduction. The balance between type I collagen (providing tissue strength) and type III collagen (contributing to elasticity) is essential for myocardial compliance (5). This balance becomes critical post-MI, as proportional reduction of both collagens may prevent excessive cardiac stiffness and preserve function. Thus, both aerobic protocols are broadly effective for myocardial improvement, though finer protocol adjustments may reveal differential effects.

These findings highlight the pivotal role of aerobic exercise—particularly HIIT—in reducing cardiac fibrosis post-MI. By effectively suppressing TGF-β (a master regulator of fibrosis), HIIT may significantly attenuate heart failure progression. This, combined with HIIT's time efficiency, makes it a practical option to enhance patient adherence. Both HIIT and MICT also reduced type I and III collagen, improving myocardial contractility (3). Thus, these results should inform cardiac rehabilitation guidelines, emphasizing HIIT to optimize recovery and reduce complications in post-MI patients. This approach may enhance cardiac health and quality of life.

While this study offers valuable insights into aerobic exercise's impact on cardiac fibrosis, several limitations warrant consideration for contextualizing results and guiding future research. First, using a rodent MI model may not fully replicate the complex physiological/molecular responses in humans (28), limiting direct clinical generalizability. Second, the duration/intensity of the protocols (HIIT and MICT) may have influenced outcomes, as parameter variations affect physiological adaptations (7). Third, precise molecular mechanisms (e.g., roles of miRNAs or other signaling pathways) were not fully explored, restricting understanding of how exercise modulates fibrotic gene expression. Finally, variables like age, sex, or comorbidities—which influence cardiac remodeling and fibrosis progression (29)—were not addressed.

To address the aforementioned limitations and expand the knowledge regarding the role of aerobic exercise in reducing cardiac fibrosis, several recommendations for future research are proposed. First, conducting studies on humans, particularly individuals with a history of myocardial infarction, is essential to confirm the effects of HIIT and MICT on cardiac fibrosis and to evaluate

their clinical applicability. Second, more detailed investigation of molecular mechanisms—such as the role of specific microRNAs (e.g., miR-29a and miR-101a) and the Smad signaling pathways involved in fibrogenesis—is recommended to gain a better understanding of the underlying processes. Additionally, testing exercise protocols with varying intensities and durations may help identify the optimal regimen for reducing cardiac fibrosis, while also taking into account patient safety and tolerance. Finally, it is recommended that the effects of exercise be examined in patients with different conditions—such as those with diabetes or older adults—to enable the personalization of treatments for various population groups.

Statements and Declarations

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Data Availability

The datasets generated during this study are available from the corresponding author upon reasonable request. This includes raw qPCR data, behavioral assessment records, and animal model parameters. Data sharing complies with institutional ethical guidelines for animal research confidentiality.

Ethics Approval

All experimental procedures involving animals were approved by the **University Ethics Committee** and performed in strict accordance with:

Consent to Participate

Not applicable. This study exclusively utilized animal models (Wistar rats) with no human participants.

References

1. Ferrero, L. (2023). *In-hospital mortality of acute myocardial infarction*. In *Epidemic Preparedness and Control*. IntechOpen. https://doi.org/10.5772/intechopen.1001453

2. Prabhu, S. D., & Frangogiannis, N. G. (2016). The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis. *Circulation Research*, 119(1), 91–112. https://doi.org/10.1161/CIRCRESAHA.116.303577

3. Talman, V., & Ruskoaho, H. (2016). Cardiac fibrosis in myocardial infarction—from repair and remodeling to regeneration. *Cell and Tissue Research*, 365, 563–581. https://doi.org/10.1007/s00441-016-2431-9

4. Jiao, H., Zhang, M., Chen, L., & Zhang, Z. (2025). Traditional Chinese Medicine targeting the TGF-β/Smad signaling pathway as a potential therapeutic strategy for renal fibrosis. Frontiers in Pharmacology, 16, 1513329.

5. Ortega, M., Fábrega-García, M. M., Molina-García, T., Gavara, J., de Dios, E., Pérez-Solé, N., ... & Ruiz-Saurí, A. (2024). Novel Fibrillar and Non-Fibrillar Collagens Involved in Fibrotic Scar Formation after Myocardial Infarction. *International Journal of Molecular Sciences*, 25(12), 6625. https://doi.org/10.3390/ijms25126625

6. Liu, J., Lu, J., Zhang, L., Liu, Y., Zhang, Y., Gao, Y., ... & Tang, Q. (2023). The combination of exercise and metformin inhibits TGF-β1/Smad pathway to attenuate myocardial fibrosis in db/db mice by reducing NF-κB–mediated inflammatory response. *Biomedicine & Pharmacotherapy*, 157, 114080. https://doi.org/10.1016/j.biopha.2022.114080

7. Ramos, J. S., Dalleck, L. C., Tjonna, A. E., Beetham, K. S., & Coombes, J. S. (2015). The impact of high-intensity interval training versus moderate-intensity continuous training on vascular function: a systematic review and meta-analysis. *Sports Medicine*, 45, 679–692. https://doi.org/10.1007/s40279-015-0321-z

8. Aispuru-Lanche, G. R., Gallego-Muñoz, M., Jayo-Montoya, J. A., Villar-Zabala, B., & Maldonado-Martín, S. (2023). Low-Volume and High-Intensity Aerobic Interval Training May Attenuate Dysfunctional Ventricular Remodeling after Myocardial Infarction: Data from the INTERFARCT Study. *Reviews in cardiovascular medicine*, *24*(1), 20. DOI: 10.31083/j.rcm2401020

9. Peyronnel, C., Kessler, J., Devaux, S. B. C., Houdayer, C., Tournier, M., Chouk, M., ... & Demougeot, C. (2024). A treadmill exercise reduced cardiac fibrosis, inflammation and vulnerability to ischemia-reperfusion in rat pristane-induced arthritis. *Life Sciences*, 341, 122503. https://doi.org/10.1016/j.lfs.2024.122503

10. Huang, C., Ding, X., Shao, J., Yang, M., Du, D., Hu, J., ... & Wan, C. (2024). Aerobic training attenuates cardiac remodeling in mice post-myocardial infarction by inhibiting the p300/CBP-associated factor. *The FASEB Journal*, 38(13), e23780. https://doi.org/10.1096/fj.202400007RR

11. Wisløff, U., Støylen, A., Loennechen, J. P., Bruvold, M., Rognmo, Ø., Haram, P. M., ... & Skjærpe, T. (2007). Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation*, 115(24), 3086–3094. https://doi.org/10.1161/CIRCULATIONAHA.106.675041

12. Li, J., Gong, L., Zhang, R., Li, S., Yu, H., Liu, Y., ... & Wang, N. (2021). Fibroblast growth factor 21 inhibited inflammation and fibrosis after myocardial infarction via EGR1. *European Journal of Pharmacology*, 910, 174470. https://doi.org/10.1016/j.ejphar.2021.174470

13. Mehdipoor, M., Damirchi, A., Razavi Tousi, S. M. T., & Babaei, P. (2021). Concurrent vitamin D supplementation and exercise training improve cardiac fibrosis via TGF-β/Smad signaling in myocardial infarction model of rats. Journal of physiology and biochemistry, 77(1), 75-84.

14. Barcelos, G. T., Rossato, D. D., Perini, J. L., Pinheiro, L. P., Carvalho, C., Jaenisch, R. B., ... & Nunes, R. B. (2017). Effects of l-arginine supplementation associated with continuous or interval aerobic training on chronic heart failure rats. *Metabolism*, 76, 1–10. https://doi.org/10.1016/j.metabol.2017.06.009

15. Nori, P., Haghshenas, R., Aftabi, Y., & Akbari, H. (2023). Comparison of moderate-intensity continuous training and high-intensity interval training effects on the Ido1-KYN-Ahr axis in the heart tissue of rats with occlusion of the left anterior descending artery. *Scientific Reports*, 13(1), 3721. https://doi.org/10.1038/s41598-023-30847-x

16. Zhang, J., Li, C., Rao, Z., Geng, X., Liang, C., Liu, S., ... & Zhao, J. (2025). Cold aerobic exercise mitigates NAFLD fibrosis through UBAP2L-regulated TGF-β/SMAD2 signaling. *Journal of Endocrinology*, 265(2). https://doi.org/10.1530/JOE-24-0351

17. Wang, S. Q., Li, D., & Yuan, Y. (2019). Long-term moderate intensity exercise alleviates myocardial fibrosis in type 2 diabetic rats via inhibitions of oxidative stress and TGF-β1/Smad pathway. *The Journal of Physiological Sciences*, 69(6), 861–873. https://doi.org/10.1007/s12576-019-00696-3

18. Zhao, Z., Zhu, Y., & Wan, D. (2025). Exercise and tissue fibrosis: recent advances in therapeutic potential and molecular mechanisms. *Frontiers in Endocrinology*, 16, 1557797. https://doi.org/10.3389/fendo.2025.1557797

19. Fu, G., Wang, Z., & Hu, S. (2024). Exercise improves cardiac fibrosis by stimulating the release of endothelial progenitor cell-derived exosomes and upregulating miR-126 expression. *Frontiers in Cardiovascular Medicine*, 11, 1323329. https://doi.org/10.3389/fcvm.2024.1323329

20. Kawanishi, N., Yano, H., Mizokami, T., Takahashi, M., Oyanagi, E., & Suzuki, K. (2012). Exercise training attenuates hepatic inflammation, fibrosis and macrophage infiltration during diet induced-obesity in mice. *Brain, Behavior, and Immunity*, 26(6), 931–941. https://doi.org/10.1016/j.bbi.2012.04.006

21. Heinemeier, K., Langberg, H., Olesen, J. L., & Kjaer, M. (2003). Role of TGF-β1 in relation to exercise-induced type I collagen synthesis in human tendinous tissue. *Journal of Applied Physiology*, 95(6), 2390–2397. https://doi.org/10.1152/japplphysiol.00403.2003

22. Nan, L., Zheng, Y., Liao, N., Li, S., Wang, Y., Chen, Z., ... & Mo, S. (2019). Mechanical force promotes the proliferation and extracellular matrix synthesis of human gingival fibroblasts cultured on 3D PLGA scaffolds via TGF-β expression. *Molecular Medicine Reports*, 19(3), 2107–2114. https://doi.org/10.3892/mmr.2019.9882

23. Ma, X., Liu, B., Jiang, Z., Rao, Z., & Zheng, L. (2025). Physical Exercise: A Promising Treatment Against Organ Fibrosis. *International Journal of Molecular Sciences*, 26(1), 343. https://doi.org/10.3390/ijms26010343

24. Xiong, Y., Wang, J., Huang, S., & Cao, Y. (2023). Investigating the effect of exercise on the expression of genes related to cardiac physiological hypertrophy. *Cellular and Molecular Biology*, 69(5), 63–69. https://doi.org/10.14715/cmb/2023.69.5.11

25. Roozbayani, M., Peeri, M., Agha-Alinejad, H., & Azarbayjani, M. A. (2016). Effect of Continues Training and High Intensity Interval Training on miR-29a and CTGF Gene Expression in Male Wistar Diabetic Rats' Heart Tissue. *Iranian Journal of Diabetes & Obesity (IJDO)*, 8(3). http://ijdo.ssu.ac.ir/article-1-310-en.html

26. Moghaddam, V., Peeri, M., Azarbayjani, M. A., & Matin Homaee, H. (2017). Comparison of the protective effect of two types of aerobic and intermittent training on breast cancer by TGFβ protein and Smad-3 gene and MMP2 in female mice. *Hormozgan Medical Journal*, 21(1), 53–64.

27. Wang, L., Chang, L., Ma, Y., Jia, Y., Gao, B., Cui, W., & Xu, F. (2025). Mechanomedicine for Addressing Skeletal Muscle Insulin Resistance. *Endocrine Reviews*, bnaf012. https://doi.org/10.1210/endrev/bnaf012

28. Bernardo, B. C., Ooi, J. Y., Weeks, K. L., Patterson, N. L., & McMullen, J. R. (2018). Understanding key mechanisms of exercise-induced cardiac protection to mitigate disease: current knowledge and emerging concepts. *Physiological Reviews*, 98(1), 419–475. https://doi.org/10.1152/physrev.00043.2016

29. Yavari, A., Najafipoor, F., Aliasgarzadeh, A., Niafar, M., & Mobasseri, M. (2012). Effect of aerobic exercise, resistance training or combined training on glycaemic control and cardiovascular