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The effect of different exercise training protocols on MMP-9 and TIMP-1 gene expression in rat myocardium

Mehran Ghahremani[1], Elham Pourrezaei[1], Saeed Naghibi[1], Afshar Jafari[2*]

1. Department of Physical Education and Sport Sciences, Physical Education and Sport Sciences, Payame Noor University (Alborz), Karaj, Iran.
2. Department of Biological Sciences in Sport, Faculty of Sport Sciences and Health, Shahid Beheshti University, Tehran, Iran (*Corresponding author: ✉ af_jafari@sbu.ac.ir, https://www.orcid.org/0000-0003-1186-4923)

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Abstract

Background: There are conflicting findings regarding the effect of various training modalities on the cardiovascular disease mechanisms induced by undesirable modifications in extracellular matrix (ECM) factors such as MMP-9 and TIMP-1.

Aim: This study was conducted to investigate the effect of different training protocols on the MMP-9 and TIMP-1 gene expression in the myocardium of Wistar male rats.

Materials and Methods: Forty-eight adult Wistar male rats (aged: 8 weeks) were randomly allocated into two control (Con8w/base, n=8; and Con16w/end control, n=8) and four training groups (MICT, n=8; PICT, n=8; HIIT, n=8; and MIST, n=8). The training protocols consisted of 8 weeks (5 days/week) of Moderate-Intensity, Continuous Training (MICT: 37 min running with 65% vVO_2max), Moderate-Intensity, Swimming Training (MIST: 30 min free-swimming), Progressive-Intensity, Continuous Training (PICT: 28 min running with a progressive increase in the incline of the treadmill by 2% per week), and High-Intensity Interval Training (HIIT: four running bouts 4 min with 90 to 100% vVO_2max + 3 min with 50-65% vVO_2max). Myocardial samples were isolated 24 hours after the last training session. Gene expression was determined using the real-time PCR method. All data (M±SD) were analyzed by one-way ANOVA and Tukey's post-hoc tests at $\alpha \leq 0.05$.

Results: There were significant differences between MMP-9 and TIMP-1 expression in the animals myocardium after different exercise training protocols ($P<0.01$). The lowest and highest MMP-9/TIMP-1 ratios were in the HIIT and MICT protocols, respectively.

Conclusion: According to our findings, the HIIT protocol may play a more influential role in enhancing myocardial ECM-remodeling and fibrosis indicators during lifespan than the other exercise protocols.

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1. Introduction

According to the World Health Organization (WHO) reports, cardiovascular diseases have been the leading cause of death worldwide over the past few decades [1]. However, most cardiovascular diseases involve severe remodeling of extracellular matrix (ECM) and detrimental modifications in ECM-anchored factors such as proteases (matrix metallopeptidases: MMPs) and proteases inhibitors (tissue inhibitors of metalloproteinases: TIMPs) [2].

Matrix metalloproteinases (MMPs) are a family of calcium- and zinc-dependent proteolytic enzymes (at least 23 endopeptidases) involved in the regeneration and degradation of extracellular matrix (ECM). MMPs participate in several biological processes, such as cell migration, growth and differentiation, tissue remodeling, and angiogenesis. In other words, matrix metalloproteinase 9 (MMP-9) can play an effective role in the angiogenesis and tissue remodeling/ fibrosis processes [3, 4, 5].

Increasing the level and activity of this protein may lead to the development of the heart remodeling process with the participation of TIMP's inhibitory proteins [6]. In other words, cross-regulation of MMPs and TIMPs proteins can play a crucial role in changing the pattern of ECM remodeling of myocardium [7, 8]. Conversely, based on the available information, TIMP-1 protein is one of the primary inhibitory proteins that have considerable effects on extracellular matrix regulation [9].

In this regard, sports scientists and researchers have studied the modifications in ECM-anchored factors (MMPs and TIMPs) in response to different exercise training protocols [10].

A review study found that exercise training protected against geometric changes of collagen ECM even in the aging heart and ameliorated age-associated dysregulation of ECM in the heart, as indicated by the up-regulation of active MMPs as well as the down-regulation of TIMPs [11].

However, the previous results are somewhat contradictory. For example, one research group reported decreased MMP-9 and increased TIMP-1 gene expression in postmenopausal women after 12 weeks of exercise training [12]. Other investigators showed that aerobic training reduces MMP-9 levels and possibly protects the heart against potential injury, while resistance training may have inconsistent results [13]. In this regard, a study showed that induction of diabetes caused a significant increase in MMP-9 by examining the effect of aerobic training on male Wistar rats in three groups (healthy control, diabetic control, and diabetic trained). TIMP-1 at the cardiac level in diabetic individuals decreased compared to the healthy group. In addition, they found that aerobic training induces significantly reduced MMP-9 and increased TIMP-1 [14].

However, the effect of various types of exercise training (with diverse modalities) on the angiogenesis mechanisms and remodeling of the ECM-anchored factors (such as MMP-9 and TIMP-1) in different tissues, especially the myocardium, is unclear and ambiguous [11, 12, 13, 14]. Therefore, to determine the preventive and therapeutic effects of various exercise training modalities on ECM-associated factors, the present study was conducted to assess the effect of four training modalities (Moderate-Intensity, Continuous Training: MICT; Progressive-Intensity, Continuous Training: PICT; High-Intensity Interval

Training: HIIT, and Moderate-Intensity, Swimming Training: MIST) on the expression of MMP-9 and TIMP-1 genes in rat myocardium.

2. Materials and Methods

2. 1. Animal (models)

According to the ethical principles outlined in the Declaration of Helsinki, in a multi-group experimental design (IR.PNU.REC.1398.045), 48 adult Wistar male rats (aged: 8 weeks, weight: 237±33 g) were randomly assigned and allocated into two control groups (Con8w/base control, n=8; and Con16w/End control, n=8) and four equal training groups (MICT, n=8; PICT, n=8; HIIT, n=8; and MIST, n=8), respectively.

The animals were in special polycarbonate cages under a 12:12-h light: dark cycle and housed at 22±2°C and 55±4% humidity, with ad libitum access to standard rat chow and fresh water. At the beginning and end of the study (before killing and dissection), it measured the body weight of the animals. Without any exercise intervention, eight- and 16-week-old control groups (Con8w/base and Con16w/end control) were killed at the beginning and end of the study, respectively.

2. 2. Procedure

The training protocols consisted of 8 weeks (5 days/week) of Moderate-Intensity, Continuous Training (MICT: running with 50-65% vVO_2max for 37 min), Progressive-Intensity, Continuous Training (PICT: running on a progressive sloping surface with 20 m/min for 37 min), High-Intensity Interval Training (HIIT: four bouts 4-min running with 90 to 100% VO_2max), and Moderate-Intensity, Swimming Training (MIST: free-swimming for 30 min). At the beginning and end of each training session, the animals ran on the treadmill at 5 m/min for 5 min warm-up and cool-down.

The MICT group ran on a treadmill with 65% vVO_2max at a 0% slope for 37 min. The rats from the MIST group continuously swam freely in a standard container swimming pool (at the laboratory temperature of 22°C) after 5 min running on a treadmill before swimming training for 30 min. The PICT group ran on a treadmill at a speed of 20 m/min with a progressive increase in the incline of the treadmill (2% per week until the eighth week to achieve 8% at the end of the intervention) for 28 min. The HIIT group performed four bouts running on a treadmill (4 min with 90-100 % vVO_2max + 3 min with 50-65% vVO_2max) for 28 min [15, 16, 17].

The animal's maximal oxygen consumption (VO_2max/vVO_2max) was estimated using a progressive exercise test (according to the protocol of Høydal et al.) which consists of running on a treadmill with 2 m/min increments every 2 min following a warm-up phase (running at 5 m/min for 10 min) [15].

The animals were anesthetized with ketamine and xylazine (100 and 10 mg/kg body weight, respectively) and killed by draining the blood from their heart. The myocardial samples of the Con8w/base and Con16w/end control groups were obtained at the beginning and end of the study design (last week of interventions), respectively. The sample tissue of the trained animals was also obtained 24 hours after the latest training session. All samples in cryotubes immediately entered the liquid nitrogen solution and were kept in the freezer at -80°C until gene expression evaluation.

The total RNA for cDNA conversion was extracted from cells using a Qiagen

tissue extraction kit (Qiagen, USA) and quantified using a spectrophotometer (DPI-Qiagen, USA) according to instructions of manufacture (Fermentas, USA). cDNA was synthesized using total RNA, oligo (dT)primers (MWG-Biotech, Germany), and RNase reverse transcriptase following the manufacturer's protocol (Fermentas, USA).

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Table 1. The sequence of primers used in the present study

Genes	Primer sequence
TIMP-1 F&R	5′- TATAGTGCTGGCTGTGGGGT-3′
	5′- GAGAAAGAAAGATGGAGGAAAGG-3′
MMP-9 F&R	5′- GACGCTGACAAGAAGTGGGG-3′
	5′- GTGGTAGTGATAGATGGGGTA-3′
GAPDH F&R	5′- GACATGCCGCCTGGAGAAAC -3′
	5′- AGCCCAGGATGCCCTTTAGT -3′

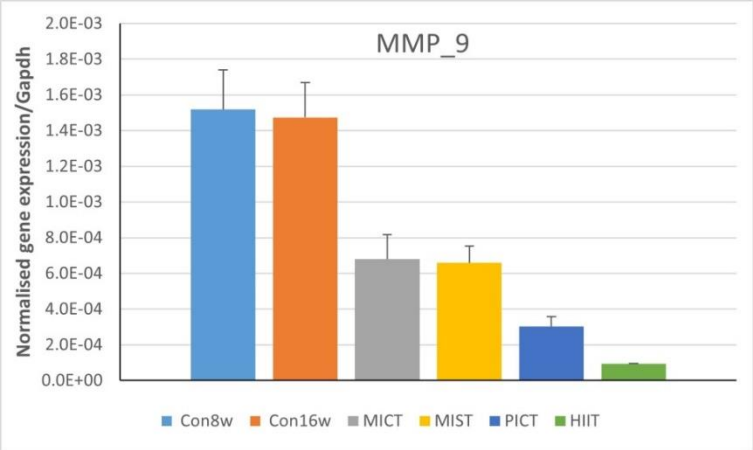

Figure 1. The MMP-9 gene expression in the left ventricular tissue of male Wistar rats

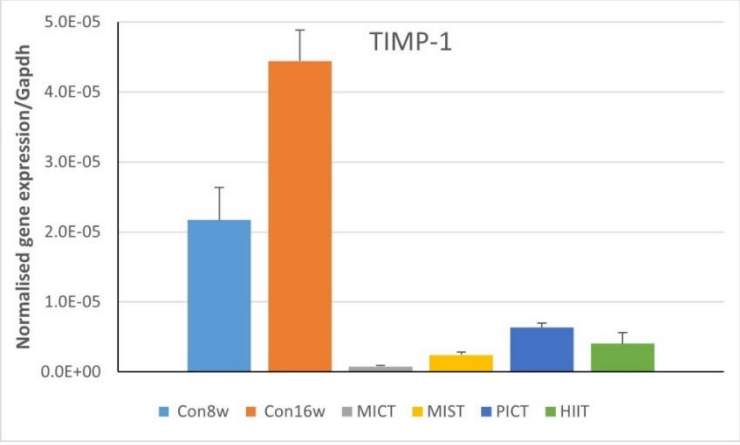

Figure 2. The TIMP-1 gene expression in the left ventricular tissue of male Wistar rats

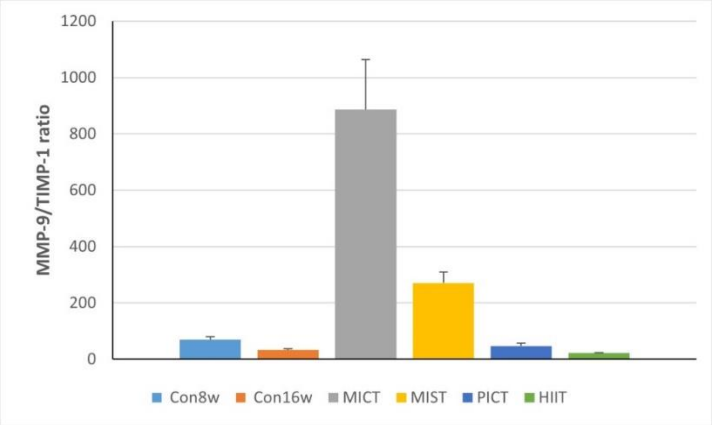

Figure 3. The expression ratio of MMP-9 to TIMP-1 genes in the left ventricular tissue of male Wistar rats

RT-PCR reaction for each mRNA was run on the ABI StepOne (Applied Biosystems, Foster City, CA) at 94°C for 10 min, followed by 40 cycles of 20 s at 94°C, 1 min at 58-60°C (MMP-9), and 30 s at 72°C. The median expression of candidate mRNAs (Ct) relative to the reference gene (GAPDH) was calculated (ΔCt). Fold changes in mRNA expression were calculated using the equation $2^{-\Delta CT}$ [18].

2. 3. Statistic

Data expressed as mean ± standard deviation (M±SD). The normality of data was assessed using the Shapiro-Wilk test, and group differences were analyzed by one-way ANOVA and Tukey's post-hoc tests (P<0.05). All statistical analyses were conducted at a significance level of 0.05, using SPSS for Windows 11.0 (SPSS Inc., Chicago, IL, USA).

3. Results

The present study results indicated the statistically significant differences between MMP-9 and TIMP-1 expression in the left ventricular (LV) myocardium of the animals that participated in various exercise training protocols (F=126.29, P<0.001 & F=275.26, P<0.001, respectively). However, the difference between the expression level of MMP-9 in the 8- and 16-week control groups was statistically insignificant (P= 0.990), but the expression level of MMP-9 in the HIIT group was significantly lower than in the other groups (P<0.001). However, the expression differences of MMP-9 in the MICT and MIST groups were not significant (P= 0.985). In other words, the lowest and highest MMP-9 expression was in experimental groups in the HIIT and MICT protocols, respectively.

There was a statistical difference between the TIMP-1 expression in the 8- and 16-week control groups (P<0.001). Furthermore, the TIMP-1 expression in the experimental groups was significantly higher than in the control groups (P<0.001). In other words, the lowest and highest TIMP-1 expression was in the HIIT and MICT groups, respectively.

The present study results indicated the statistically significant differences between the ratio of MMP-9 to TIMP-1 in the LV myocardium of the animals that participated in different training protocols (F=78.52, P<0.001), despite the MMP-9/TIMP-1 ratio difference between two control groups (at the beginning and end of the intervention) was not significant (P<0.05). However, the rate of this indicator in the MICT group was significantly higher than in the control and other experimental groups (P<0.001),

although the ratio of MMP-9 to TIMP-1 in the HIIT group was lower than in other groups (P<0.001).

4. Discussion

The present study results confirm some of the previous findings that indicate aging may affect some indicators of extracellular matrix turnover. In our study, the expression level of the TIMP-1 gene in the Con16w group was higher than in the Con8w group (P<0.001), but the difference between the MMP-9 gene expression level and MMP-9/ TIMP-1 ratio in the Con16w/end control group compared to the Con8w/base control group was not statistically significant (P<0.05). In this regard, some previous findings showed that aging reduces the MMP-9 gene expression while it induces an increase in TIMP expression and a reduction of extracellular matrix accumulation [16]. Our results also indicate that MMP-9 gene expression in all trained experimental groups is lower than in the untrained control groups at the beginning and end of the intervention. In other words, participating in the different types of training may reduce the MMP-9 gene expression. The reduction rate of the gene expression (the lowest rate in the HIIT group) was higher in the training protocols with high intensity (with more mechanical-metabolic stress). However, the tendency changes in TIMP-1 gene expression are slightly different. In this regard, we observed the lowest possible rate in the MICT group. Furthermore, the highest and lowest change ranges in the MMP-9/ TIMP-1 ratio were in the MICT and HIIT groups, respectively.

The increasing alterations in MMP-9 expression and the MMP-9/ TIMP-1 ratio after the MICT and MIST protocols might be due to the moderate intensity of these types of exercise training; because the lowest TIMP-1 expression and MMP-9/ TIMP-1 ratio were in the HIIT group, whereas the MMP-9 to TIMP-1 ratio in the MICT group was significantly higher than the other experimental groups. Consistent with our findings, some previous results indicate a decline in myocardial tissue MMP-9 expression after high-intensity exercise training. For example, an early study showed a decrease in MMP-9 gene expression after an eight-week HIIT protocol in 30 women with breast cancer [17]. One possible explanation for these observations is that MMP-9 expression is elevated by oxidative stress suggesting that the oxidative stress molecules may increase MMP-9 synthesis. It is possible that HIIT training caused a more pronounced reduction in oxidative stress in the ECM which subsequently reduced MMP-9 levels. The oxidative stress induced by high-intensity exercise (HIT or HIIT with intensity higher than 80% of VO_2max) may stimulate cardiac fibroblast proliferation and activate the matrix metalloproteinases, leading to extracellular matrix remodeling [19].

However, studies showed a decrease in MMP-9 occurs after 8 weeks of running (60 min at 25 m/min) or 6 weeks of resistance training [20], while the other results revealed that 8-week endurance and resistance training (5 days per week) increases the MMP-9 expression [21]. It seems multiple factors such as duration, intensity, volume, and frequency of physical exercises, types of tissue, MMP-9 concentrations at baseline, and sampling time may affect the response of these indicators to different kinds of exercise protocols [22].

For example, some previous findings showed elevations in MMP-9 levels up to 3

days after exercise training [23]. Therefore, the acute elevation in MMP-9 response to different exercise protocols may continue for up to a few days. Consequently, it should consider an appropriate interval to eliminate the acute effects of exercise protocols when examining the long-term results of training courses on these indicators.

5. Conclusions

According to the ratio of MMP-9 to TIMP-1, the high-intensity interval training (HIIT) protocol's effects on angiogenesis and myocardial ECM remodeling during lifespan may be better than the other training modalities. In other words, the HIIT protocols might protect against heart ECM deterioration and fibrosis. Nevertheless, further study is necessary to determine the protective effect of different exercise training targets to mitigate fibrosis and cardiovascular disease.

Conflict of interest

The authors declared no conflicts of interest.

Authors' contributions

All authors contributed to analysis of the results and to the writing of the manuscript.

Ethical considerations

The authors have completely considered ethical issues, including informed consent, plagiarism, data fabrication, misconduct, and/or falsification, double publication and/or redundancy, submission, etc. The project was found to be in accordance to the ethical principles and the national norms and standards for conducting Medical Research in Iran: IR.PNU.REC.1398.045.

Data availability

The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

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