Original Article

Improvement of Angiogenic Biomarkers Following Aerobic Training in Rats With Experimental Myocardial Infarction

Mozhgan Ashtari[1], PhD; Bahram Abedi[1*], PhD; Hoseyn Fatolahi[2], PhD

ABSTRACT

Background: The present study aimed to compare 4 weeks of exercise (EXE) on the plasma levels of angiogenic markers in male and female rats with experimental myocardial infarction (MI).

Methods: Forty male and female Wistar rats were randomly and equally divided into 4 groups: male MI, male MI + EXE, female MI, and female MI + EXE. Subcutaneous injection of isoproterenol (150 mg/kg) was used to induce MI. The training groups performed an endurance training protocol on the treadmill for 4 weeks (speed=18 m/min, 30 min, 5 sessions per week). Sacrifice and blood sampling were performed 24 hours after the last training session. The data were analyzed using 2-way ANOVA and LSD *post hoc* tests.

Results: Vascular endothelial growth factor levels were significantly higher in the female MI + EXE group than in the male MI + EXE group (P=0.037). Changes in matrix metalloproteinase 2 levels were greater in the female rat group than in the male rat group (P=0.017). Matrix metalloproteinase 9 levels were higher in the MI + EXE female rats than in the MI + EXE male rats (P=0.008).

Conclusions: Our findings showed that sports rehabilitation had positive effects on experimental MI, which is likely to cause clinical changes and improve angiogenic parameters in female rats more than in male rats. ***(Iranian Heart Journal 2022; 23(3): 24-32)***

KEYWORDS: Angiogenic biomarkers, Rats, Myocardial infarction, Isoproterenol, Aerobic Training

[1] Department of Physical Education and Sports Sciences, Mahallat Branch, Islamic Azad University, Mahallat, IR Iran.
[2] Department of Physical Education, Pardis Branch, Islamic Azad University, Pardis, IR Iran.

***Corresponding Author:** Bahram Abedi, PhD; Department of Physical Education and Sports Sciences, Mahallat Branch, Islamic Azad University, Mahallat, IR Iran.
Email: abedi@iaumahallat.ac.ir **Tel:** +989188667662

Received: December 11, 2020 **Accepted:** November 3, 2021

Cardiovascular diseases are the leading cause of death in modern societies, and myocardial infarction (MI) is one of the most common causes.[1] Some studies have stated that sex may be the cause of differences observed following MI in men and women.[2] Angiogenesis is significant in cardiovascular events, including MI. The active reproduction process of endothelial cells, the formation of active blood vessels, and the growth and development of new blood vessels via the germination of endothelial cells of previous vessels are called angiogenesis.[3] Therapeutic angiogenesis is a new strategy

used to treat tissue ischemia by increasing the proliferation of parallel vessels. This strategy was developed as a significant treatment strategy for ischemic diseases.[4] In adults, this process is inherent in response to myocardial hypoxia and occurs even in the absence of hypoxia. The decreased partial pressure of oxygen due to exercise (EXE) is one of the most important causes of angiogenesis, and it is seen in the upstream regulation of vascular endothelial growth factor (VEGF).[3]

In ischemic tissue, hypoxia-induced angiogenesis occurs due to the increased expression of some growth factors. VEGF is a glycoprotein cytokine responsible for a set of events leading to vascular formation during physiological and pathological processes. Changes in VEGF levels serve as a major factor in angiogenesis. Consistent with these positive effects, some drugs used in rats with MI are effective in modulating inflammatory mediators and concomitant increases in pro-antigenic factors such as VEGF. Therefore, low VEGF levels are an independent risk factor for adverse clinical outcomes after MI.[5]

Atherosclerosis is a progressive inflammatory process in which migrating cells secrete more inflammatory cytokines and matrix metalloproteinases (MMPs).[6] In the first 30 minutes after MI, the extracellular matrix (ECM) is destroyed by the release of inflammatory mediators and the production of metalloproteinases.[7] MMPs belong to the family of endogenous enzymes, interstitial collagenase, and depending on zinc and calcium, they are responsible for ECM regeneration in several physiological and pathological processes. Therefore, MMP2 and MMP9 levels significantly increase in the period after MI and play an important role in myocardial regeneration, collagenase, and inflammatory messages. Indeed, the amount of circulating MMPs plays an important role in cardiovascular diseases as a potential biomarker.[8] The activation of MMPs leads to plaque deformation, which is directly involved in plaque rupture. Additionally, MMPs are involved in cardiac deformity following MI and dilated cardiomyopathy development, and the circulation levels of MMPs are now considered a potential biomarker to determine cardiovascular risks for plaque rupture and coronary risks.[9]

Hence, the upregulation of angiogenic factors such as VEGF and the stimulation of anti-inflammatory factors for managing cardiovascular disorders such as MI are deemed significant today.[10] Human and animal studies have demonstrated that one of the factors influencing these processes is EXE.[11,12] EXE is a multifactorial stimulus that can stimulate metabolic and mechanical stimuli to initiate angiogenesis response. Mechanical forces during muscle activity such as shear stress, passive tension, and messages from metabolic changes such as decreased oxygen delivery lead to the increased expression and secretion of some pro-angiogenic agents and angiostatic elements, resulting in the onset of capillary growth.[13] Therefore, the effects of endurance training on the growth of new blood vessels can be examined based on the type and intensity of training. Three mechanisms of visceral fat reduction, the release of contracted anti-inflammatory muscle cytokines, and the decreased expression of toll-like receptors of monocytes and macrophages have been suggested as the anti-inflammatory mechanisms of EXE.[14] Moreover, three months of EXE (3 sessions per week) improves resting stored heart rate recovery and EXE capacity.[15] Therefore, EXE before MI not only helps prevent and increase fitness but is also important for accelerating recovery after a complication.[4] Furthermore, the positive effects of EXE on MI may be sex-dependent. In this regard, Brown et al[16] (2005) showed that the heart of female rats was inherently more resistant to ischemic injury than that of male rats. Maleki et al[3] (2019) observed an increase in serum MMP levels

and an improvement in the process of angiogenesis in male rats with MI following endurance training. It is essential to study the effects of EXE based on Sex. Accordingly, the present study aimed to investigate the effects of 4 weeks of endurance training on the plasma levels of angiogenic markers in male and female rats with experimental MI.

METHODS

Samples and Research Environment

The present study has an experimental research design (posttest with a control group). The experimental protocol was approved by the Ethics Committee of Islamic Azad University, Science and Research Branch (No. IR.IAU.SRB.REC.1398.062). The study was performed following the guidelines of the National Institutes of Health for the care and use of laboratory animals (No. 80-23).

The statistical population of the study consisted of male and female Wistar rats aged 12 to 14 weeks with a mean weight of 250 to 300 g. Forty animals (20 male and 20 female rats) were selected as the statistical sample. The animals were randomly divided into 4 groups: male MI, male MI + EXE, female MI, and female MI + EXE. Before the experiments, the animals were kept in polycarbonate cages for 2 weeks under standard conditions of 12/12 hour light-dark cycles, temperatures of 22±3 °C, and humidity of 50±5%. With the aid of 2 silent air conditioners, the status of air pollutants was set at the desired level of the Pollutant Standard Index (PSI).[17] The rats freely accessed water and food (Pars Food Company, Tehran, Iran).

MI Induction Protocol

For the induction of MI, isoproterenol (Sigma-Aldrich, USA) (150 mg/kg) was injected subcutaneously [18] For the measurement of isoproterenol, a digital Sartorius laboratory scale was used with an accuracy of one-tenth of a gram of the element. This substance was used in a diluted normal saline solution (0.05 mL) for 2 consecutive days at an interval of 24 hours. Forty-eight hours after the last injection, 2 rats from each group were randomly selected and subjected to experimental conditions for MI induction. MI was confirmed based on clinical signs, electrocardiographic (GE Healthcare, USA) changes, and elevations in cardiac enzymes such as cardiac troponin I. The level of cardiac troponin I was 294/168 pg/mL in the rats with MI, whereas no elevation in cardiac troponin I was detected in the healthy animals. Troponin I has the highest susceptibility to myocardial cell damage and is a major diagnostic method.[19] In addition, an elevation in the ST segment of the electrocardiogram of the rats with MI (Fig. 1B) compared with the healthy rats (Fig. 1A) indicated MI occurrence.

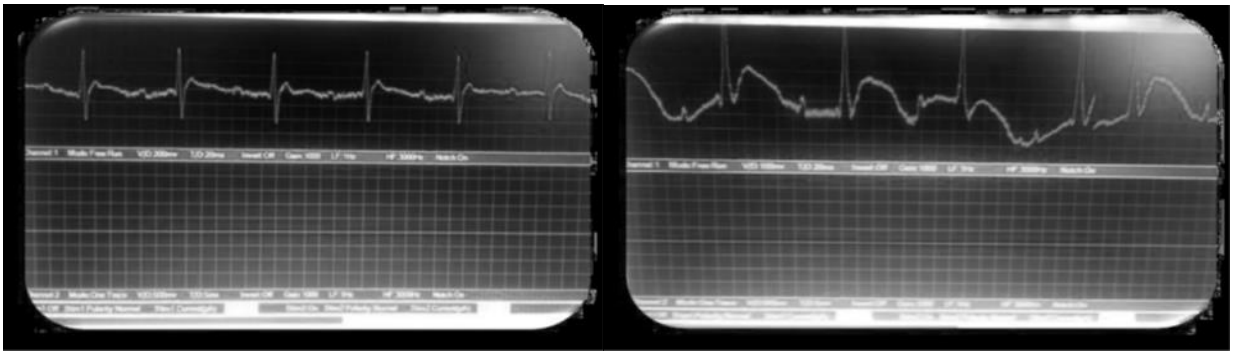

Figure 1: Section A illustrates the electrocardiogram of a healthy rat, and section B illustrates the electrocardiogram of a rat with myocardial infarction.

Endurance Training Protocol

The animals were kept in the new conditions for 2 weeks. In the second week, all the rats learned how to work on the treadmill. The introduction program was performed for 1 week, 10 minutes a day, at a speed of 8 meters per minute without slope (0% slope) on a treadmill (Bionic-Mobin Company, DSI-580).[17] The main program of the EXE groups included 4 weeks of running on the treadmill at a speed of 18 meters per minute for 30 minutes, 5 times a week. The training program was performed at a 0° slope, and it was accompanied by warming up and cooling down for 3 minutes at a speed of 8 meters per minute. The control groups did not exercise during the experiment and were kept in cages.[20]

Blood Collection and Biochemical Assays

Forty-eight hours after the last training session, the animals were anesthetized and sacrificed with a mixture of ketamine and xylazine (75 and 10 mg/kg, respectively). Blood sampling was done after direct anesthesia from the right atrium of the rats' hearts with 10 mL tubular syringes. The collected blood was poured into a clot activator serum tube and subjected to ambient temperature for 10 minutes. The serum was separated by centrifugation (Hermle Z200A, Germany) for 5 minutes at 5000 rpm. Then, the plasma levels of VEGF, MMP2, and MMP9 were taken via the ELISA method based on the instructions of the manufacturer of the animal model kits (East Biopharm Company) (inter-assay and intra-assay change coefficients were <10, 1.4, and 8%, and the sensitivity levels of the measurement methods were 0.05 and 0.2 ng/mL and 7.8 pg/mL, respectively).

Statistical Analysis

Descriptive statistics were used to determine the mean and the standard deviation of the data. The Kolmogorov–Smirnov test was employed to determine the normality of data distribution. Two-way ANOVA was applied to investigate the independent and interactive effects of sex and EXE. If significant differences were observed between the groups, the location of differences was determined using the LSD *post hoc* test. The Pearson correlation test was also utilized to describe the relationships between intergroup indices. The data were analyzed using SPSS 24, and statistical significance was considered at a *P* value of 0.05 or less.

RESULTS

The mean and the standard deviation of the study variables in the studied groups are presented in Table 1.

Table 1: Mean and the standard deviation of the studied variables

Variables/Groups	Female+MI	Female+MI+EXE	Male+ MI	Male+ MI+EXE
Weight(kg)	249.87±16.02	260.1±14.79	313.87±15.8	**300.62±12.52**
VEGF (ng/mL)	88.7±12.05	94.53±9.36	82.59±6.73	**85.68±11.38**
MMP2 (ng/mL)	14.93±4.89	12.7±2.5	10.38±1.45	**12.62±3.41**
MMP9 (ng/mL)	1.5±0.45	1.77±0.22	1.49±0.25	**1.22±0.43**

Table 2: Results of the Pearson correlation coefficient test

Variables and Groups	Correlation Levels	Variables and Groups
VEGF: Female+MI	$P = 0.004$, $r = 0.918$	MMP2: Female+MI group
VEGF: Female+MI	$P = 0.022$, $r = 0.827$	MMP9: Female+MI
VEGF: Male + MI	$P = 0.018$, $r = 0.841$	VEGF: Male + MI +EXE
VEGF: Male + MI	$P = 0.048$, $r = 0.758$	MMP9: Male+MI + EXE

VEGF: Male +MI + EXE	*P* = 0.005, *r* = 0.905	MMP2: Male +MI + EXE
VEGF: Male +MI + EXE	*P* = 0.005, *r* = 0.908	MMP9: Male +MI + EXE
MMP2: Female+ MI	*P* = 0.008, *r* = 0.885	MMP9: Female+MI
MMP2: Female+ MI	*P* = 0.022, *r* = 0.826	MMP2: Male +MI
MMP2: Male+ MI + EXE	*P* = 0.005, *r* = 0.907	MMP9: Male+MI + EXE

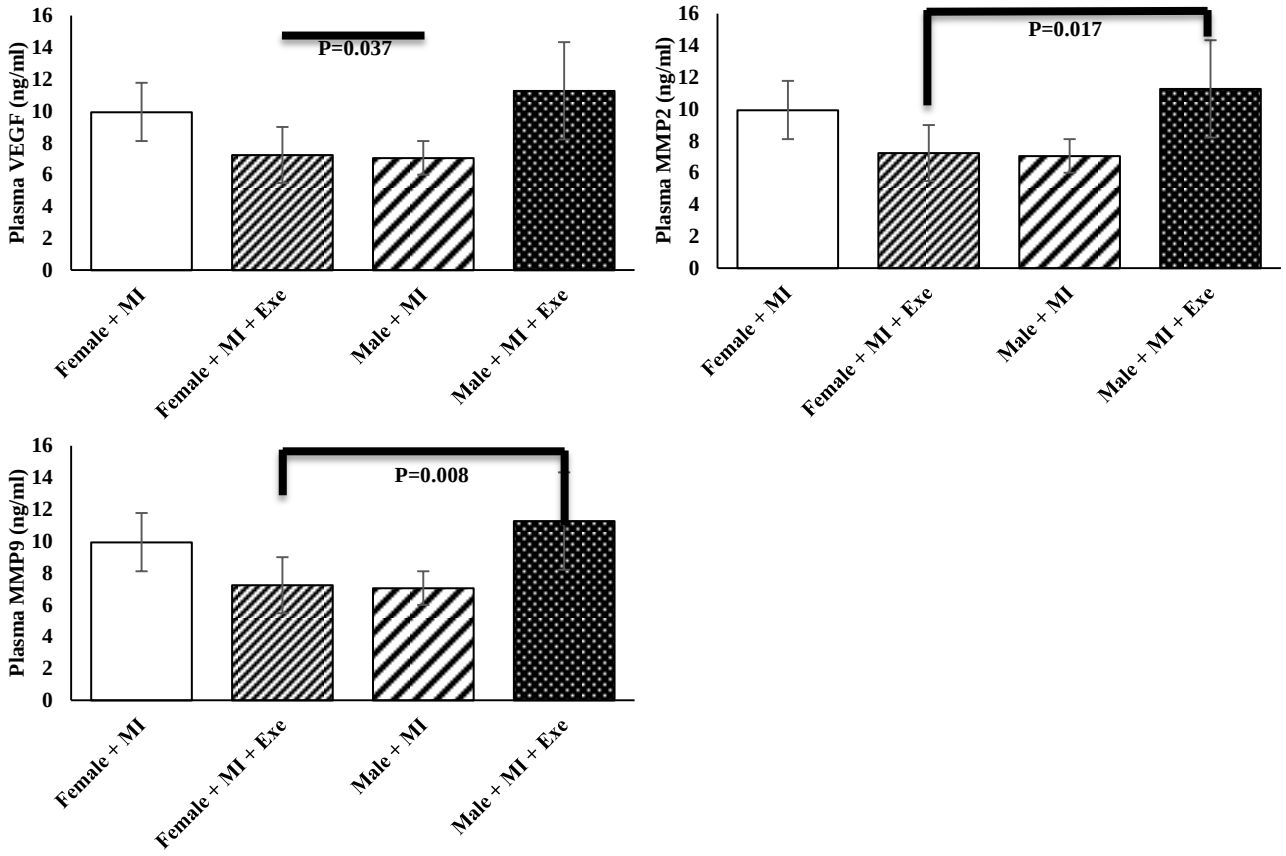

Figure 2: The figures illustrate changes in angiogenic variables, including VEGF (Section A), MMP2 (Section B), and MMP9 (Section C) in response to exercise rehabilitation exercise taking into account the effect of sex in male and female Wistar rats.
Female rats + experimental infarction induction (Female + MI), female rats + experimental infarction induction + rehabilitation exercises (Female + MI + Exe), male rats + experimental infarction induction (Male + MI), male rats + infarction induction Experimental + Rehabilitation Exercises (Male + MI + Exe)

The results of the 2-way analysis of the variance test showed that 4 weeks of endurance training had no significant interactive effect on VEGF levels in the male and female rats (*P*=0.732, F=0.129, μ=0.005). However, rehabilitation and sex had a significant independent effect on changes in the VEGF index (*P*=0.042, F=13.84, μ=0.438). The results of the *post hoc* test showed that VEGF values were significantly higher in the Female+MI+EXE group than in the Male+MI group (*P*=0.037) (Fig. 2: Section A).

The results of the 2-way analysis of the variance test showed that endurance training did not have a significant interactive effect on MMP2 levels in the male and female rats (*P*= 0.995, F=0.001, μ=0.001). On the other hand, the effect of sex on MMP2 was borderline significant (*P*=0.07, F=17.42, μ=0.425). The results of the *post hoc* test showed that higher values of MMP2 were observed in the female

rat group than in the male rat group after MI (*P*=0.017) (Fig. 2: Section B).

The results of the 2-way analysis of the variance test revealed that the effects of endurance training on MMP9 in the male and female rats with experimental MI were not significantly different (*P*=0.92, F=0.091, μ=0.051). Moreover, the results indicated the lack of an interactive effect for the studied variables on MMP9 (*P*=0.062, F=3.902, μ=0.14). Nonetheless, an independent effect for sex was observed (*P*=0.047, F=14.375, μ=0.354). The results of the *post hoc* test demonstrated higher MMP9 values in the Female MI+EXE group than in the Male MI+EXE group (*P* = 0.008) (Fig. 2: Section C).

The results of the Pearson correlation coefficient test (Table 2) demonstrated relationships between VEGF in the Female+MI group, with MMP2 in the Female+MI group (*P* = 0.004, *r* = 0.918) and MMP9 in the Female+MI group (*P* = 0.022, *r* = 0.827). Further, there was a relationship between VEGF in the Male+MI group and the Male+MI+EXE group (*P*=0.018, *r*=0.841) and MMP9 in the Male+MI+EXE group (*P*=0.048, *r*=0.758). VEGF in the Male+MI+EXE group had a significant positive relationship with MMP2 in the Male+MI+EXE group (*P*=0.005, *r*=0.905) and MMP9 in the Male+MI+EXE group (*P*=0.005, *r*=0.908). A relationship was also observed concerning MMP2 changes in the Female+MI group, with MMP9 in the Female+MI group (*P*=0.008, *r*=0.885) and MMP2 in the Male+MI group (*P*=0.022, *r*=0.826). MMP2 in the Male+MI+EXE group had a positive and direct relationship with MMP9 in the Male+MI +EXE group (*P*=0.005, *r*=0.907).

DISCUSSION

The present study aimed to evaluate the effects of 4 weeks of rehabilitation endurance training on the serum levels of VEGF, MMP2, and MMP9 in male and female rats with experimental MI. No interactive effects were observed; still, in all the cases, the response of female rats to rehabilitation EXE was significantly better. Comparing the effects of 8 weeks of aerobic and resistance training on angiogenic factors in inactive women, Toloui Azar et al[22] (2019) showed that both types of training significantly increased serum VEGF levels. Karbalaeifar et al[21] (2019) investigated the effects of high-intensity interval training (6 weeks, 60 minutes of treadmill running, each cycle consisting of 4 minutes with an intensity equivalent to 90–85% VO_{2max}, 4 days a week) in rats with MI. Their results showed that VEGF expression was significantly higher in the EXE group than in the control group. Jazi et al[23] reported that endurance training had no significant effects on VEGF expression. Contrary to these studies, Wu et al[24] (2009) showed that EXE in mice with MI increased the expression of VEGF and its receptors.

EXE-induced shear stress is one of the most notable mechanisms that can improve vascular function.[25] Factors such as the intensity, duration, and type of EXE, as well as different experimental methods, may play a role in the different effects of EXE after MI.[26] The intensity and duration of endurance training in the present study may not have been sufficient to stimulate VEGF expression, although MI was induced in the animals. A nonsignificant increase in biomarkers cannot necessarily be correlated with the effects of EXE. Research has shown that in the immediate wake of MI, serum VEGF levels increase, indicating the beginning of the regeneration process. Therefore, regular physical activity and effective diet plans could speed up rehabilitation. On the other hand, significant differences between experimental groups can be attributed to high VEGF in female rats compared with male rats.[27]

Acute MI causes tissue damage, inflammation, and elevations in MMP2 and MMP9, triggering rapid regeneration after injury.

Another reason for the increase in MMPs is an adaptation to EXE, which initiates the destruction of basal endothelial tissue and increases capillary secretion and blood flow to the heart by secreting VEGF. Finally, after proper recovery and adaptation to EXE, MMP levels are restored to rest values.[24] Among the inflammatory factors expressed, proteinases are the last link in a large chain of immune and inflammatory mediators in response to different types of EXE.[3] Moreover, a lack of significant change in the activity of MMP2 and MMP9 can reduce tissue damage and cause a significant rise in VEGF, as one of the main factors of angiogenesis. Training protocols should lead to tissue destruction via MMPs and regeneration of the ECM through myofibroblast regulation by establishing a balanced signal pathway. Changes should occur in the serum levels of MMPs due to increased expressions in skeletal muscle cells and endothelial cells. Alterations in the levels of MMPs in the bloodstream indicate changes in their tissue levels.[9] Regarding sex differences, Ranjbar et al [28] (2015) assessed MMP2 and MMP9 levels in men and women at rest and after 1 endurance activity with an intensity of 70% VO_{2max} and showed that the average concentration of MMP2 in the control and experimental groups was proportional.

Scientific reports consider the increase of VEGF and the MMP2/MMP9 ratio through MAPK and the PI3K/Akt signaling pathway and under the influence of visfatin as important factors in angiogenesis. Increasing each of the capillary factors in its physiological range is valuable because in cases such as diabetic patients with cardiovascular complications and the growth of cancerous tumors, this issue finds another aspect. Therefore, the role of regular physical activity to control the disease is like a double-edged sword.[6] For instance, in a study of the effects of endurance training on MMP2 and MMP9 levels in postmenopausal women with hypertension, researchers found that the levels of this index decreased significantly.[29] However, if we regard inflammation and oxidative stress as some of the most important factors and preconditions for diseases, especially MI, regular EXE is one of the best ways to prevent them. Extensive studies of patients with MI have demonstrated improvements after moderate-intensity regular physical activity programs.[14] Nevertheless, our findings regarding changes in MMPs and VEGFs in response to endurance training may have many reasons.

Changes in the tissue blood flow due to selected physical activity, the rate of adaptation to EXE, the period of inactivity, the pattern of EXE (duration and intensity), and the measured tissue are important factors concerning changes in these variables. In this regard, researchers refer to the design of a special type of EXE in cardiovascular patients called “ischemic EXE”, one of the main mechanisms of which is VEGF stimulation.[30]

CONCLUSIONS

Overall, the results of the present study showed that 4 weeks of an endurance training program caused significant changes in the inflammatory and angiogenic indices of male and female rats with experimental MI. The change was more pronounced in female rats. Since the beneficial effects of endurance training on MI may be associated with a decrease in inflammatory markers and changes in angiogenic markers, further research is needed on the effectiveness of these activities by controlling other disruptive factors. To confirm these results, we need more studies considering the effect of sex. Perhaps one of the best ways to handle this is to use a diet plan and anti-inflammatory foods along with physical activity.

Disclosure

The corresponding author acknowledges that there is no conflict of interest among the authors. All the authors have read and

contributed equally to the final text of the article.

Acknowledgments
This article is taken from a PhD dissertation (code: 20021423981005). The authors of the present article express their gratitude to all those who assisted in the research.

Authors' Contributions
M. A. and B. A.: conceptualization, methodology, investigation, resources, data acquisition, visualization, supervision, project administration, and funding acquisition. H. F.: software, validation, formal analysis, writing the original draft preparation, and writing, editing, and reviewing

REFERENCES

1. Zhang R, Lan C, Pei H, Duan G, Huang L, Li L. Expression of circulating miR-486 and miR-150 in patients with acute myocardial infarction. BMC cardiovascular disorders. 2015;15(1):51.

2. Chen W, Woods SL, Puntillo KA. Gender differences in symptoms associated with acute myocardial infarction: a review of the research. Heart & Lung. 2005;34(4):240-7.

3. Maleki Poya M, Abedi B, Palizvan M, Saremi A. The Effect of Eight Weeks of Incremental Endurance Training on The Levels of Matrix Metalloproteinase-1 (MMP1) and Thrombosponidine-1 (TSP1) in the Rats, Induced by Myocardial Infarction by Isoproterenol. Arak University of Medical Sciences. 2019;22(3):118-28.

4. Freedman SB, Isner JM. Therapeutic angiogenesis for ischemic cardiovascular disease. Journal of molecular and cellular cardiology. 2001;33(3):379-93.

5. Niu J, Han X, Qi H, Yin J, Zhang Z, Zhang Z. Correlation between vascular endothelial growth factor and long-term prognosis in patients with acute myocardial infarction. Experimental and therapeutic medicine. 2016;12(1):475-9.

6. Hoke M, Winter M-P, Wagner O, Exner M, Schillinger M, Arnold Z, et al. The impact of selectins on mortality in stable carotid atherosclerosis. Thrombosis and haemostasis. 2015;114(09):632-8.

7. Ma Y, de Castro Brás LE, Toba H, Iyer RP, Hall ME, Winniford MD, et al. Myofibroblasts and the extracellular matrix network in post-myocardial infarction cardiac remodeling. Pflügers Archiv-European Journal of Physiology. 2014; 466(6):1113-27.

8. Murillo CA, Woodside KJ, Guo Q, Zhang S, O'Connor KL, Hunter GC. Integrin and matrix metalloproteinase expression in human carotid plaque. Journal of Surgical Research. 2009; 155(1):157-64.

9. Liu P, Sun M, Sader S. Matrix metalloproteinases in cardiovascular disease. Canadian Journal of Cardiology. 2006; 22:25B-30B.

10. Christia P, Frangogiannis NG. Targeting inflammatory pathways in myocardial infarction. European journal of clinical investigation. 2013;43(9):986-95.

11. Maleki MJ, Fatolahi H, Feylizadeh A, Abedi B. The effect of rehabilitation training and total checkup as a noninvasive method on hemodynamic parameters in patients who candidate for heart transplantation. International Archives of Health Sciences. 2019;6(1):24-9.

12. Rahmati-Ahmadabad S, Azarbayjani M-A, Farzanegi P, Moradi L. High-intensity interval training has a greater effect on reverse cholesterol transport elements compared with moderate-intensity continuous training in obese male rats. European Journal of Preventive Cardiology. 2019:2047487319887828.

13. Haas TL, Nwadozi E. Regulation of skeletal muscle capillary growth in exercise and disease. Applied Physiology, Nutrition, and Metabolism. 2015;40(12):1221-32.

14. Pedersen BK. Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. European journal of clinical investigation. 2017;47(8):600-11.

15. Elshazly A, Khorshid H, Hanna H, Ali A. Effect of exercise training on heart rate recovery in

patients post anterior myocardial infarction. The Egyptian Heart Journal. 2018;70(4):283-5.

16. Brown DA, Lynch JM, Armstrong CJ, Caruso NM, Ehlers LB, Johnson MS, et al. Susceptibility of the heart to ischaemia–reperfusion injury and exercise-induced cardioprotection are sex-dependent in the rat. The Journal of physiology. 2005;564(2):619-30.

17. Lloyd PG, Prior BM, Yang HT, Terjung RL. Angiogenic growth factor expression in rat skeletal muscle in response to exercise training. American Journal of Physiology-Heart and Circulatory Physiology. 2003; 284(5):H1668-H78.

18. Bertinchant JP, Robert E, Polge A, Marty-Double C, Fabbro-Peray P, Poirey S, et al. Comparison of the diagnostic value of cardiac troponin I and T determinations for detecting early myocardial damage and the relationship with histological findings after isoprenaline-induced cardiac injury in rats. Clinica Chimica Acta. 2000;298(1):13-28.

19. Sun Y, Qi G, Li D, Meng H, Zhu Z, Zhao Y, et al. Walnut (Juglans regia L.) Kernel Extracts Protect Against Isoproterenol-Induced Myocardial Infarction in Rats. Rejuvenation research. 2019;22(4):306-12.

20. Alimohammadi R, Naderi S, Allahtavakoli M. Effects of Exercise and Estrogen on Anxiety-like Behaviors in Ovariectomized Mice. Journal of Babol University of Medical Sciences. 2015;17(12):40-6.

21. Karbalaeifar S, Gaeini AA, Kordi MR, Nuri R, Ghorbani P. Effect of 6 Weeks High Intensity Interval Training on Selected Factors of Cardiac Angiogenesis in Rats with Myocardial Infarction. Sport Physiology. 2019; 11(42):17-30.

22. Tolouei Azar J, Ravasi A, soori R, Akbarnejat A, Hemati Nafar M. The Comparison of the Effect of 8 Weeks of Aerobic and Resistance Training on Angiogenic (VEGF) and Angiostatic (ES) Factors in Sedentary Women. Journal of Sport Biosciences. 2019;11(1):1-16.

23. Azamian Jazi A, Haffezi MR, Opera H, Abdi H. The effect of endurance exercise training and atorvastatin on VEGF in rat following experimental myocardial infarction. scientific journal of ilam university of medical sciences. 2016; 24(4):21-31.

24. Wu G, Rana JS, Wykrzykowska J, Du Z, Ke Q, Kang P, et al. Exercise-induced expression of VEGF and salvation of myocardium in the early stage of myocardial infarction. American Journal of Physiology-Heart and Circulatory Physiology. 2009; 296(2):H389-H95.

25. Rodríguez I, González M. Physiological mechanisms of vascular response induced by shear stress and effect of exercise in systemic and placental circulation. Frontiers in pharmacology. 2014; 5:209.

26. Wan W, Powers AS, Li J, Zhang JQ, Ji L, Erikson JM. Effect of Post–Myocardial Infarction Exercise Training on the Renin-Angiotensin-Aldosterone System and Cardiac Function. The American journal of the medical sciences. 2007;334(4):265-73.

27. Malamitsi-Puchner A, Tziotis MD J, Tsonou MSc A, Protonotariou E, Sarandakou A, Creatsas G. Changes in Serum Levels of Vascular Endothelial Growth Factor in Males and Females Throughout Life. The Journal of the Society for Gynecologic Investigation. 2000;7(309-312).

28. Ranjbar K, Nazem F, Gholami MR, Nezami AR, Ardakanizade M, Sohrabi M, et al. Synergistic effects of nitric oxide and exercise on revascularisation in the infarcted ventricle in a murine model of myocardial infarction. PMC. 2015;14(EXCLI J):1104-015.

29. Bordbarazari B, Gholami M, Ebrahim K, Abed Natanzi H, Ghazalian F. The effect of endurance training along with L-arginine supplementation on the levels of MMP-2 and MMP-9 in postmenopausal hypertensive women. Journal of Basic Research in Medical Sciences. 2019;6(4):20-8.

30. Gao M, Lu X, Chen W, Xiao GH, Zhang Y, Yu R, et al. Randomized clinical trial of physiological ischemic training for patients with coronary heart disease complicated with heart failure: Safety of training, VEGF of peripheral blood and quality of life. Experimental and therapeutic medicine. 2018;16(1):260-4.