



Effect of Continuous Endurance Training on mRNA Expression of Interferon Gamma and Matrix Metalloproteinase-9 in Male Elder Rats

Elham Sadat Mousavi ¹, Mohammad Reza Asad ¹, Ali Barzegari ^{1,*} and Mahbobeh Gholizadeh Ahangari ¹

¹Department of Physical Education and Sport, Payame Noor University, Tehran, Iran

*Corresponding author: Department of Physical Education and Sport, Payame Noor University, Tehran, Iran. Tel: +98-911130029, Fax: +98-1132250048, Email: ali_barzegari@pnu.ac.ir

Received 2018 December 09; Accepted 2018 December 09.

Abstract

Background: Aging impairs function of the heart and is associated with mechanical remodeling. This process involves accumulation of collagen and dysfunction in the regulation of active matrix metalloproteinase. On the other hand, exercise training improves cardiac function and regulates the increase of pro-inflammatory molecules, such as interferon gamma, yet the impact of exercise training on aging has not yet been defined.

Objectives: This study examined the effect of continuous endurance training on mRNA expression of interferon gamma (IFN- γ) and matrix metalloproteinase-9 (MMP-9) in male elder rats.

Methods: For this purpose, 14 Wistar male rats with age of 24 to 26 months and an average weight of 380 ± 20 g were randomly divided to two groups, including training ($n = 7$) and control ($n = 7$). Training groups performed continuous endurance training for six weeks. Matrix metalloproteinase-9 mRNA and interferon (IFN)- γ mRNA expression in heart tissue were measured by real-time polymerase chain reaction. To analyze of the data, the independent *t*-test was used at a significance level of $P < 0.05$.

Results: The findings of this statistical analysis showed that six weeks of continuous endurance training had a significant effect on MMP-9 mRNA expression ($P = 0.001$). Also, this training protocol did not have a significant effect on expression of IFN- γ gene ($P = 0.628$).

Conclusions: Continuous endurance training attenuated aging-induced cardiac inflammation of rats. There are numerous questions that remain to be answered to complete the understanding of the moderator effects of exercise on MMP-9 and IFN- γ , as a muscle conformity or indicate an inflammatory condition.

Keywords: Endurance-Continuous Training, Interferon-Gamma, Matrix Metalloproteinase-9, Elder Rats

1. Background

Almost all living organisms experience a progressive deterioration of their physiological functions over time, which leads to an increase in their susceptibility to disease and an increased risk of death (1). In this regard, one of the significant aspects in relation to aging is the disruption of cardiac function of older individuals (2). Across a broad range of species, one consistent hallmark of cardiac aging is a decrease in myocardial reserve capacity (3). Cardiac aging by itself results in a slight yet significant decline in left ventricle (LV) function (4). In conditions related to aging of the heart, cardiomyocytes release pro-inflammatory cytokines that stimulate an immune response to increase macrophage numbers in the LV (5). Macrophages are a rich source of matrix metalloproteinase (MMPs), and an unbalanced MMP activity profile has been linked to myocardial

aging status in humans with no evidence of cardiovascular disease (6) across a variety of animal models (7). Matrix metalloproteinase are one of the most important families of proteases involved in the tight control of extracellular matrix (ECM) remodeling over time (2). Among the MMPs that increase along with the aging of the heart, MMP-9 was considered by the researchers of this study. In association with elevated MMP-9, macrophages increase in an age-dependent manner to regulate ECM and angiogenic responses. There is strong evidence that MMP-9 is a major mediator for increased stiffness in aging LV. Furthermore, MMP-9 is predominantly expressed in leukocytes, with low expression in cardiomyocytes (8). Macrophage-derived MMP-9 has been implicated in cardiac aging, so that MMP-9 expression increases by two folds in the LV of aged mice (5).

One of the major regulators of MMP-9 production in extracellular matrix and their endogenous inhibition through inflammatory cells, such as circulating monocytes and macrophages, is interferon gamma (IFN- γ). According to performed experiments on human subjects with coronary artery disease, it has been shown that IFN- γ levels in these patients are higher than healthy subjects, which results in an imbalance in the ratio of MMP-9 to its specific inhibitor (TIMP-1). It has been observed that IFN- γ increases the incidence of heart disease by increasing the expression of MMP-9 in human subjects (9).

One of the most important objectives of public health is to decrease age-related disabilities among the elderly. In this regard, appropriate physical activity for the elderly can be used to prevent, delay, or treat problems caused by aging. As noted, aging develops chronic inflammation. Exercise also effects inflammatory conditions. Regular exercise, particularly endurance exercise, effectively improves heart function in older populations. It is possible that exercise training in the aging population may reduce accumulation of connective tissue (10). Although, limited data indicates that exercise training might attenuate collagen content in the aging heart, results on the relationship between exercise training and IFN- γ and MMP-9 expression after training is inconsistent. In this regard, Farinha et al. reported that following 12 weeks of treadmill exercise training in untrained women with metabolic syndrome, the serum levels of IFN- γ was decreased in peripheral blood mononuclear cells (11). Silva et al., after four weeks of exercise training running (ExT) (60 minutes/day, five days/week) of male Wistar rats showed decreased MMP-9 mRNA levels and MMP-2 expression compared to sedentary control rats. They declared that reduction in MMP-9 mRNA expression may constitute an underlying mechanism, by which ExT counteracts progression of adverse LV remodeling in T1D (12). Moreover, Novaes et al. demonstrated that cardiac and serum levels of IFN- γ increased in Wistar rats after nine weeks of treadmill running training (13), while Reihmane et al. found an increase in MMP-9 levels, immediately after exercise in marathon runners (14).

According to the effect of IFN- γ in response to inflammation and stimulation of the immune system (15), as well as regulating of expression of mRNA MMP-9 (9), on one hand and the multiple roles of MMP-9 in the remodeling of cardiac ECM (2) and response to inflammatory conditions in aging (5), it seems that one of the mechanisms, through which exercise can modulate inflammatory responses and delay cardiac dysfunction of older individuals is evaluating the expression of the MMP-9, IFN- γ gene.

2. Objectives

All these contradictory results, as well as the absence of a study that has evaluated these two factors in the elderly led to the development of a specific exercise protocol that evaluates the expression of mRNA MMP-9 and IFN- γ in elderly rats.

3. Methods

3.1. Animals

The present experimental study was performed at Tarbiat Modares University. Fourteen Wistar rats with age of 24 to 26 months and an average weight of 380 ± 20 g were purchased from the Pasteur Institute of Iran. After two weeks of becoming familiar with the environment and the research protocol, animals were divided randomly to two groups that consisted of training (n=7) and control groups (n = 7). Animals in an environment with an average temperature of $22 \pm 3^\circ\text{C}$, humidity of 45%, and light dark cycle of 12:12 hours were kept in cages made of polycarbonate. Keeping animals was carried out in accordance with the International Health Institute instructions, and this study was approved by the HRI Ethics Committee of Payam Noor University (code No.: IR.PNU.REC.1397.031).

3.2. Experimental Design

All animals were habituated with a motor-driven treadmill (Tarbiat Modarres University lab; Iran) for two weeks (16, 17), 10 minutes/day, and 10 to 18 m/minute. Following familiarization, all animals, 48 hours after the last session, accomplished an additive test to overtiredness on a treadmill (beginning at 10 m/minute with augmentations of three m/minute every two minutes) (18). Overtiredness was demarcated as the incapacity of the rats to run on the treadmill regardless of small electric shocks and failure to straight themselves when located on their backs.

3.3. Training Intervention

As shown in Table 1, after determining the peak speed (30 m/minute), the rats performed the training program five times/week for six weeks. Continuous training (running on treadmill) started with an intensity of 65% of peak speed and increased from the third week to 5%. Then the speed was stabilized from the third to the sixth week. The training duration started from 15 minutes then, five minutes was added to the training time, each week. Finally, from the fourth to the sixth week the training was stabilized (Table 1). The research design was post-test and consisted of two independent groups, each consisting of an experimental group (training) and a control group, and the subjects were randomly divided to these groups.

Table 1. Summary of the Training During the Experimental Period

Training Weeks	Average Training Intensity Per Session, %	Duration of Each Session (Min)	Training Per Week (Min)
1	65	15	75
2	65	20	100
3	70	25	125
4	70	30	150
5	70	30	150
6	70	30	150

To eliminate the effect of acute exercise, sampling of the animals was performed 48 hours after the last session of training. Then, animals were anesthetized via intraperitoneal injection of ketamine (30 to 50 mg/kg) and xylazine (3 to 5 mg/kg). After thoracic surgery, heart tissue was isolated and placed in microtubes and frizzed in liquid nitrogen at -70°C. The cDNA synthesis kit produced by the Thermo Scientific with catalog number K1 622 was used in this study. RNA extraction and cDNA synthesis were performed in accordance with the standard protocols of the manufacturer. In order to extract RNA and synthesize cDNA, about 50 mg of muscle tissue was separated and homogenized to extract total RNA from 1 to 10 in QIAzol Lysis Reagent.

3.4. Detection of MMP-9 mRNA and IFN-γ mRNA Expression Using Quantitative Real Time PCR

Expression of MMP-9 mRNA and IFN-γ mRNA was measured by PCR. Each PCR reaction, by using PCR master mix and SYBRGreen (Applied Biosystems), was performed on the “ABI step one” device (Applied Biosystems, Sequence Detection Systems, Foster city, USA), according to the manufacturer’s instruction. Total RNA (4 μg) extracted from heart tissues were reverse transcribed to cDNA using murine leukemia virus reverse transcriptase (Applied Biosystems, Foster city, CA). The resulting cDNA was diluted by 1:30 fold and the PCR reaction was performed with 2.5 μL of cDNA, 0.2 μM of each forward and reverse primers, 12.5 μL of SYBR Green PCR Master Mix (Applied Biosystems) in a final volume of 25 μL. The thermal profile for the real-time Q-PCR was 50°C for two minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for one minute. The gene expression was expressed as fold change from the GAPDH level, which was calculated as $2^{-\Delta\Delta Ct}$. In addition, melting curve analysis was performed to assure the specificity of PCR product in this experiment. First, the mRNA sequence of the MMP-9 and IFN-γ gene was extracted using the NCBI site. The primers were made by the AllelID software, and then each primer was evaluated by the BLAST software, to ensure the placement of coupling

of primers was unique. Primers were made by the Cinnagen Company. In this research, the GAPDH gene was applied as an interior control. The sequence of forward and reverse primers for GAPDH genes was used as the reference gene. Forty cycles were considered for each cycle of real-time PCR and each cycle’s temperature was set to 94°C for 15 seconds, and 60°C for 30 seconds. For all genes considered, the GAPDH gene was also used to obtain the annealing temperature gradient. The primers of rats are listed in Table 2.

Table 2. Primer Sequences and Size of Products of Target Genes

Transcript	Sequence (5’→3’)
MMP-9 (F)	5’AAACTGGATGACAATGCTCTGC3’
MMP-9 (R)	5’CGAAGGGCAGCTCAAGTG3’
IFN-γ (F)	5’GATCCAGCACAAAAGCTGTCA3’
IFN-γ (R)	5’GACTCCTTTTCCGCTTCCTT3’

Abbreviations: F, forward primer; R, reverse primer.

3.5. Statistical Analysis

In order to determine the normal distribution of data and homogeneity of variance, the current research used Shapiro-Wilk test and Leven’s test, respectively. Then, to analyze the data, independent *t*-test were used at a significance level of $P < 0.05$ to investigate changes of IFN-γ and MMP-9 expression. Mean and standard deviation were used to report the values of the measured variable. All statistical analyses were executed using the SPSS statistical software package (version 24.0).

4. Results

The current findings did not show significant changes in body weight (control group: 360 ± 20 g, training group: 380 ± 21 g) of the research groups. It should be noted that the weight of the subjects was measured before and after the implementation of the protocol exercise ($P = 0.121$). The results of independent *t*-test also noted that after a

period of continuous endurance training, no significant changes were observed in IFN- γ mRNA expression compared to control ($P = 0.128$) (Figure 1). In addition, the findings showed that MMP-9 mRNA expression following continuous endurance training had a significant increase when compared to the control (21.32%) ($P = 0.001$) (Figure 2).

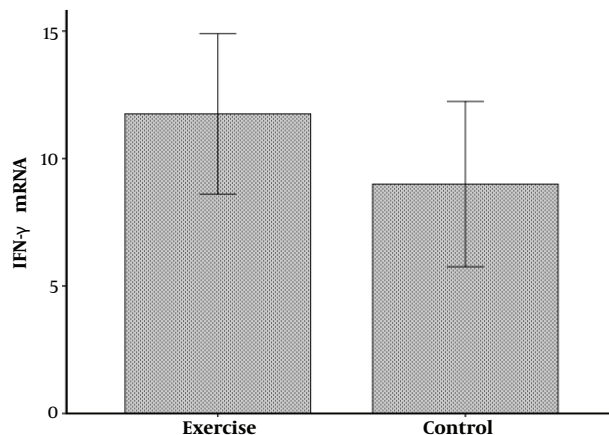


Figure 1. Changes in IFN- γ mRNA of research groups. Training ($n = 7$) and control ($n = 7$). Independent t -test were used at a significance level of $P < 0.05$ to investigate changes of IFN- γ mRNA expression.

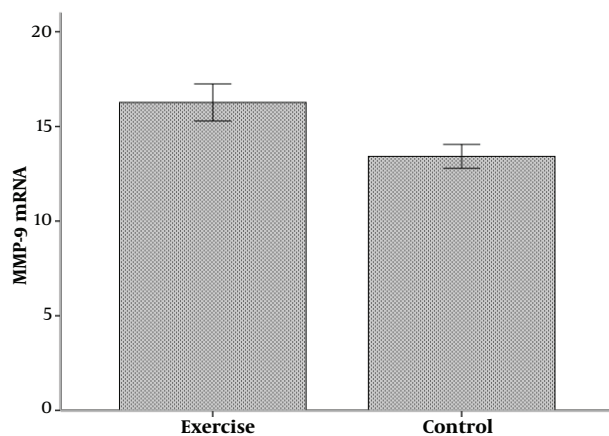


Figure 2. Changes in MMP-9 mRNA expression in heart tissue of research groups. Training ($n = 7$) and control ($n = 7$). * Signs of a significant changes compared to control. Independent t -test were used at a significance level of $P < 0.05$ to investigate changes of MMP-9 mRNA expression.

5. Discussion

The purpose of this research was to examine the effect of continuous tolerance training on mRNA expression of

IFN- γ and MMP-9 in male elder rats. The findings showed that six weeks of continuous endurance training had a significant effect on MMP-9 gene expression in the training groups. Consistent with the findings of the current study, Rullman et al. (19) indicated that following a bout of exercise, the expression of MMP-9 mRNA and motivated MMP-9 protein in skeletal muscle increased. Regulation of mRNA is observed as a vital procedure for alterations in MMP-9 function. It has been suggested that sic transcriptional stimulation is derived by muscle injury and inciting reactions. As they observed increased activity of MMP-9 mRNA, 120 minutes after exercise, they expressed that in exercised muscle, the quantity of inflammatory cells is insignificant, even since a sole struggle of difficult eccentric execution, the expression of numerous cytokines likewise IL-1 α , and tumor necrosis factor-alpha (TNF- α) are recognized to rise in the skeletal muscle tissue. These phagocytes have been displayed to rise MMP-9 mRNA levels and consequently were probable entrant for adapting the increase in MMP-9 transcription (19).

Nascimento et al. investigated the influence of dissimilar exercise intermediation on MMP-9 in human investigations. In diseased circumstances like metabolic disorder and coronary risk, the enhancement of MMP-9 was associated with amplified inflammation. Therefore, less amount of inflammatory indicators might moderate the repressive effect of exercise on MMP-9 levels. The reduction in MMP-9 and MMP-2, as a result of persistent exercise training, could be associated with expression of their innermost protein suppressor, like TIMPs, α 2-macroglobulin, and protease demolition. Furthermore, substrate accessibility and convenience characterize the level that MMP-9 activity were applied. One more probable mechanism is the decrement of TNF- α induced by the training, a well-known exciter of MMP-9 production. Results revealed that chronic aerobic training leads to cardio protective effects and is useful for the prevention of diabetes, hypertension, cancer, osteoporosis, and dementia (20).

Moreover, results showed that continuous exercise training showed no significant deference in IFN- γ gene expression when compared to the control group. In this regard, Sugama et al. compared changes in the plasma and urinary levels of cytokines after endurance exercise and observed that IFN- γ did not change significantly after training as compared with the pre-exercise values, whereas urinary excretion of IFN- γ increased significantly. Therefore, it might be possible that IFN- γ was produced in the kidney. This data suggests that although some cytokines may not appear in the circulation during exercise, they appeared in the urine after several hours following endurance exercise, which indicated their production and/or clearance from the bloodstream in the kidney. These findings might

help develop reliable biomarkers of immunity and inflammation for the assessment of endurance exercise workload as well as the pathological mechanisms of strenuous exercise (21). Of course, this finding is consistent with the unexplained changes of the current study, yet the measurements had not been at the level of gene expression.

In contrast, Sotoodeh Jahromi et al. reported that following the performance of a particular training protocol (running on a treadmill for 15 to 30 minutes at 50% to 70% maximal heart rate) during eight weeks in healthy young males, showed significant decreases in the serum IFN- γ concentration. They expressed that the creation of IFN- γ from T cells was suppressed by cortisol and epinephrine, which were enhanced in response to training. Consequently, this mechanism can be important for the reduction of IFN- γ levels. The reaction of cytokines to training was multiplex and associated with the intensity of the training, exercise situations, site of cytokine evaluation (e.g. tissue, plasma or urine), and sensibility of measurement process (22). This study wasn't consistent with the current study and this difference can be due to differences in subjects, measurement methods, and differences in exercise protocols. However, in this study, many variables, such as species, race, gender, weight, environmental factors (sound, light, humidity, temperature), and food control were under control. Nevertheless, the present study included limitations, such as the lack of control of nighttime activities of the subjects and possible interference with the results of the research and the lack of control caused by the injection of anesthetic substance. In conclusion, there is obvious technical confirmation of the profits of training for the inhibition of cardiovascular disorders. However, the decrease of MMP-9 and IFN- γ levels was weakened by training in individuals with diseased conditions and might show the cardio protective result of training; there are numerous problems in recognizing the moderate effects of training on MMP-9 and IFN- γ , and their importance as a muscle conformity or demonstrator of an inflammatory state.

Supplementary Material

Supplementary material(s) is available [here](#) [To read supplementary materials, please refer to the journal website and open PDF/HTML].

Acknowledgments

The authors gratefully thank the Tarbiat Modares University for supporting this study.

Footnotes

Conflict of Interests: The authors declare no conflict of interest.

Ethical Considerations: The experimental procedures were approved by the Tarbiat Modares University Ethics Committee (code No.: IR.PNU.REC.1397.031).

Funding/Support: None declared.

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