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The Effect of Eight Weeks of Continuous Endurance Training on ICAM-1 and VCAM-1 Expression in the Heart Tissue of Rats

M.R. Asad (PhD)[1], M. Sistani (MSc)[1], A. Barzegari (PhD)[*1]

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

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ABSTRACT

BACKGROUND AND OBJECTIVE: The expression of adhesion molecules in the heart tissue is considered as an important indicator in the estimation of coronary artery disease risk. Since physical activity is effective in reducing symptoms of cardiac disease, the purpose of the present study was to investigate the effect of eight weeks of continuous endurance training on ICAM-1 and VCAM-1 expression in the heart tissue of rats.

METHODS: In this experimental study, 12 eight weeks male Wistar rats with an average weight of 263±12 g were randomly divided into 2 groups: control (n=6) and exercise (n=6) were divided. The training program included 8 weeks of running on a treadmill with a 70 to 75% VO2max intensity (five days a week and 30 minutes each day). The expression of ICAM-1 and VCAM-1 genes were measured by Real-time PCR and compared in two groups.

FINDINGS: The results showed that 8 weeks of continuous endurance training reduced the expression of ICAM-1 in the heart tissue. In comparison, there was a significant difference between the control group (0.29±0.05) and training (0.10±0.03) (p=0.017). In addition, it was shown that the expression of VCAM-1 in the heart tissue significantly increased in training group (0.85±0.04) compared to the control group (0.22±0.03) (p=0.001).

CONCLUSION: The results of this study showed that continuous endurance training altered the expression of adhesion molecules in heart tissue associated with systemic inflammation, which could be a suitable solution for reducing fat deposits in the heart tissue and preventing its harm.

KEY WORDS: *Endurance Training, Rats, Intercellular Adhesion Molecule-1, Vascular Cell Adhesion Molecule-1.*

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[*] Corresonding Author: A. Barzegari (PhD)

Address: Department of Physical Education and Sport, Payame Noor University, Tehran, I.R.Iran

Tel: +98 21 84234102

E-mail: ali_barzegari@pnu.ac.ir

Introduction

Cardiovascular disease is a major cause of death in developed countries, and according to various studies, its prevalence is increasing in our country (1,2). Many risk factors such as hypertension, diabetes, obesity, inheritance, and poor lifestyle have been reported among adolescents and middle-aged people (3), so identifying and reducing cardiovascular risk factors can have important clinical applications (4). Studies have shown that the spread of cardiovascular disease, including atherosclerosis, is inflammatory (5-7). Much evidence has shown that cell adhesion molecules play an important role in cardiovascular pathogenesis, and new markers including ICAM-1 (Intercellular Adhesion Molecule 1) and VCAM-1 (Vascular Cell Adhesion Molecule-1) genes are more sensitive and accurate for prediction and detection of the risk of cardiovascular events and play an important role in the process of atherosclerosis (7-9).

These cell adhesion molecules are a key member of the immunoglobulins present at the endothelial surface and regulate leukocyte responses to inflammation by binding to different integrins (10). Therefore, any action that reduces inflammatory markers is likely to reduce cardiovascular events (11). Previous studies have confirmed the positive effect of exercise on reducing adhesion molecules in subjects with coronary heart disease (12,13). As cardiovascular disease and mortality due to it are increasing day by day, providing essential strategies such as determining the type of exercise, its duration and severity to reduce the incidence of heart disease seems necessary (14).

In this regard, Mogharnasi et al showed that 12 weeks of treadmill running significantly reduced ICAM-1 in rats (15). However, Signorelli et al reported that after a period of exercise training for people with arterial problems, serum levels of ICAM-1 were elevated (16). Adamopoulos et al found that 12 weeks of pedunculation at 50 to 80% of maximal heart rate was associated with a significant decrease in VCAM-1 in patients with heart failure (13). However, Signorelli et al reported that after a period of exercise training in subjects with arterial problems, serum VCAM-1 levels were elevated (16). In another study, after 14 weeks of aerobic exercise in healthy women, no significant change in plasma ICAM-1 and VCAM-1 was reported (17). Exercise appears to be able to reduce the risk of cardiovascular disease by controlling the factors involved in blood pressure and inflammation, which require studies at the cellular and tissue levels. Since the VCAM-1 and ICAM-1 are of particular importance in the diagnosis of heart disease, the attainment of appropriate exercise practices and the varying intensity of exercises have been of interest to physiology researchers in recent years, and these factors have been studied in cardiac tissue following endurance training. Although possible mechanisms have been suggested, the results of studies on the relationship between physical activity and the expression of ICAM-1 and VCAM-1 genes are inconsistent (16,17).

Given the association between ICAM-1 and VCAM-1 gene expression with cardiovascular disease and the supportive role of exercise in coping with it and the lack of research on high-intensity continuous training, this study aimed to investigate the effect of 8 weeks of continuous endurance training on the expression of ICAM-1 and VCAM-1 genes in male rat heart tissue to investigate the effect of adaptation to endurance training on adhesion molecule function.

Methods

Animals: This experimental study after approval by the Ethics Committee of Payame Noor University with code IR.PNU.1397.031 was performed on 12 male Wistar 8-week-old rats weighing 263+12 g, purchased from Razi Institute. The rats were randomly divided into two groups of six (control and training groups) after two weeks of familiarization with the environment and the research protocol. Animals were kept in specially polycarbonate cages at an average temperature of 22±4.1°C, 55±4% humidity, and 12:12 h light and dark cycle.

Exercise program: The exercise protocol consisted of eight continuous endurance exercises, five sessions per week and 30 minutes of running each session with an intensity of 70 to 75% of maximal oxygen consumption with a slope of zero on the treadmill (18). At the end of the two weeks of familiarization, the rats' maximal oxygen uptake (VO2max) was measured and the protocol started five sessions of exercise per week. At the end of every two weeks, the VO2max test was performed and a new training speed was considered for the week following exercise. Due to the lack of access

to direct respiratory gas analysis tools, the aerobic capacity of mice was investigated using the research of Høydal et al. (19). Exercise started with 10 min warm-up at 40-50% VO2max intensity and after warm-up, the main training protocol was initially started at 15 m/min for two minutes. Then the treadmill speed was increased once every two minutes by 0.03 m/s (1.8 to 2 m/min). The training protocol time was 30 minutes and the intensity of training was 70-75% VO2max. After the end of the training protocol, the cooling activity was performed for 10 minutes at 40-50% VO2max intensity. Animals in the control group were similar to those in the exercise group except for daily training and, for simulation, were trained on treadmill at 2 m/min for 15 minutes three times a week for 15 minutes each (20).

Experimental tests: Animals were sampled 48 h after the last training session to eliminate the acute effect of training. For this purpose, the animals were anesthetized and killed by peritoneal injection of ketamine (50-30 mg/kg) and xylazine (3-5 mg/kg). After rupturing the chest, the heart muscle was separated, washed in saline, and immediately frozen using liquid nitrogen and transferred to a freezer at -80 C for subsequent assays. RNA was extracted using 50 mg left ventricle of the heart. Tissue was lysated with 1 mM Trizol solution and homogenized with a tissue homogenizer. Then, the aqueous phase was separated by 0.25 ml of chloroform. The extracted RNA was washed and dried with 1 ml of 70% cold ethanol and then sterile water (1.5 L/mg tissue) was added. The quantitative measurement of extracted RNA was performed using a 260 nm bio filter. The cDNA was extracted for each sample during three cDNA synthesis steps. Thus, eight micrograms of RNA were first mixed with 0.8 ml DNase I enzyme, 2 ml buffer 10x and DEPC water and the sample volume was reduced to 20 ml.

The resulting product was slowly mixed without vortexing and then incubated in the thermocycler according to schedule. After completion of the thermocycler stages, 280 ml of injection water was added and stored at -20 C for use in QPCR. For each cDNA sample, a positive control sample with b2m primer as internal control was prepared to test for cDNA presence. The primers used in this study are listed in Table 1. PCR Time-Real PCR was performed on animal tissue RNAs using One Step SYBR Prime Script RT-PCR kit produced by Takara company and Corbet real-time PCR produced by Roror gene company. The reaction mixture was prepared in a final volume of 22 ml. An internal control was also provided for each sample on the homologous polymerase gene. The primers were designed based on the nucleotide sequence of the VCAM-1 and ICAM-1 genes in the NBCI GenBank using Oligo7 software and synthesized by Macrogen corporation. After the synthesis, the graphs were analyzed and the expression changes of genes were calculated using ΔΔCT (analysis based on CT difference between intervention groups).

Statistical methods: Data were analyzed by SPSS software using Kolmogorov-Smirnov (KS) test and independent t-test and $p<0.05$ was considered significant.

Table 1. Primer used in research

Gene	Host	Forward primer (5'–3')	Reverse primer (5'–3')
ICAM-1	Rat	tttgcaagaaaagccaacatgaaag	tctccaacagttcagacgttagc
VCAM-1	Rat	agatcatacgggtttgggcttc	tatgactcgtgaaagaaatcagctc

Results

At the beginning of the study, the body weight values of the rats in the control group (210.5±5/11 g) and the exercise group (235.23±8.38 g) were not significantly different. After eight weeks of continuous endurance training, weights of rats reached 225.5±18.12 g and 230.19±8.35 g, respectively, which were not statistically significant. Also, the results of independent t-test showed that eight weeks of endurance training decreased (65.51%) ICAM-1 mRNA expression in cardiac tissue in the exercise group which showed a significant difference between the control group (0.29±0.05 pg/ml) and exercise group (0.00±10.3 pg/ml) ($p = 0.017$) (Fig 1). In addition, it was shown that eight weeks of continuous endurance training significantly increased cardiac tissue VCAM-1 mRNA expression (286.65%) in exercise group (0.00±85.04 pg/ml) compared to control group (0.22±0.03 pg/ml) ($p=0.001$) (Fig 2).

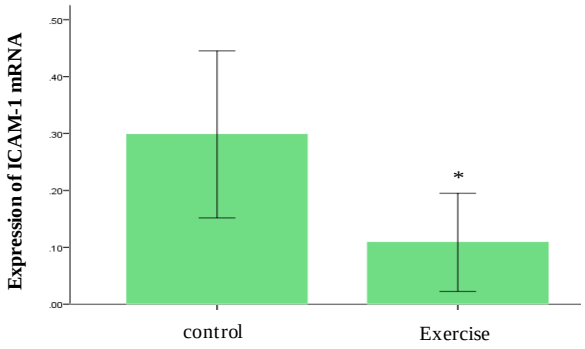

Figure 1. Comparison of ICAM-1 mRNA expression of cardiac tissue of the research groups. Significance level was considered as p<0.05.

*** indicates significant difference than the control group**

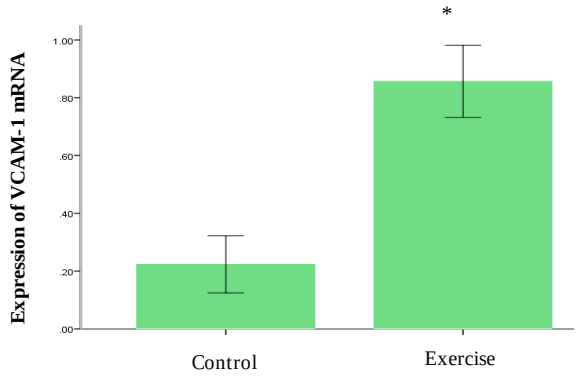

Figure 2. Comparison of VCAM-1 mRNA expression of cardiac tissue of the research groups. Significance level was considered as p<0.05.

*** indicates significant difference compared to control group**

Discussion

The results of the present study showed that a period of endurance training program significantly decreased ICAM-1 expression and significantly increased VCAM-1 expression of cardiac tissue of rats in comparison with control group. Consistent with the results of the present study, Mogharnasi et al. found that serum levels of ICAM-1 in male rats decreased significantly after the endurance training protocol (21). Regular exercise activities appear to inhibit inflammatory mediators of adipose tissue and, in turn, decrease ICAM-1 concentrations by reducing sympathetic stimulation and elevating anti-inflammatory cytokines (22).

Another mechanism in reducing the inflammatory marker ICAM-1 may be the antioxidant effects of aerobic exercise. Since free radicals increase the expression of the inflammatory mediator ICAM-1, aerobic exercise can reduce the inflammatory markers by enhancing antioxidant defense (23). Jalaly and colleagues also observed a significant decrease in ICAM-1 after 12 weeks of aerobic exercise in patients with angina pectoris (24). High-intensity endurance activity reduces TNF-α production and since release of adhesion molecules is stimulated by increased secretion of inflammatory cytokines, reduction of pro-inflammatory factors decreases release of chemical mediators including NF -kB which can be effective in modulating inflammation. NF-kB is inactive in the cytoplasm and mediates translation of ICAM-1 (22).

According to the evidence, a decrease in shear stress leads to a decrease in ICAM-1 levels and its release from vascular endothelial cells (25). On the other hand, Brevetti et al., reported increased levels of ICAM-1 adhesion molecules after maximal activity, which is inconsistent with the results of the current study. Differences in the results of this study can be due to differences in subject type, training protocol, length of study period or amount of confounding factors. Brevetti et al.'s research sample had intermittent tremor and their practice protocol was considered to be acute (26). In general, the mechanisms of expression of adhesion molecules following exercise training may depend on the amount of exercise activity, intensity, duration, and repetition of the training sessions. Based on the results of this study, it was observed that eight weeks of continuous endurance training significantly increased VCAM-1 expression in rat heart tissue. Consistent with the present study, Bartzeliotou et al reported that serum VCAM-1 levels increase after the end of acute inflammation induced by prolonged exercise (27). The mechanism of VCAM-1 enhancement is related to stimulation of growth factors. Most invasion of circulating cells into the tissue depends on the extracellular matrix-degrading proteases and, as a result of physical activity, matrix metalloproteinase levels increase. Therefore, physical activity as a stimulus in remodeling can lead to the use of circulating cells (28). Nielsen et al. also stated that in both groups of marathon and half marathon runners, the levels of cell adhesion molecules were significantly increased after the race. These changes have a negative effect on leukocyte adhesion and also increase leukocyte activity for migration from endothelium to tissues (29). On the other hand, Sarderoodian et al showed that after eight weeks of baseline exercise and 4 sessions per week for

50 minutes with 60 to 75% of maximal oxygen saturation, no significant effect was observed on VCAM-1 levels in postmenopausal women with hypertension (30).

Mogharnasi et al. also showed that aerobic exercise with 55 to 85 percent of maximal oxygen consumption resulted in a significant reduction in adhesion molecules, However, adhesion molecules expression returned to baseline levels after 4 weeks of de-training. The differences in the findings of the reported studies may be due to differences in subject type, exercise protocol, and duration of the study(31). A swimming exercise protocol was used in the Sarderoodian study and the study samples were postmenopausal women with hypertension (30). In fact, aerobic exercise can reduce inflammatory indexes by enhancing anti-oxidative mechanisms and reducing free radicals (32). The reason for the significant increase in VCAM-1 appears to be the short duration of training. It should be noted, however, that according to the research, this adhesion molecule is less affected by exercise than ICAM-1. Limitations of the present study include the short duration of training and the inability to control physical activity outside the research program. The results of this study showed that endurance training decreased ICAM-1 expression and increased VCAM-1 expression. Given the information available in this area, the effect of continuous endurance training on adhesion molecules cannot be determined with certainty. However, with the increasing incidence of cardiovascular disorders in different age groups, continuous endurance training can be a viable solution to reduce fat deposits in the heart tissue and prevent its damage.

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