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The effect of eight weeks aerobic exercise on troponin T and metallothionein levels of cardiac tissue in healthy male rats

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Abstract

Introduction: Cardiac Troponin T (cTnT) is currently considered as an ideal biomarker for the diagnosis of cardiac injury and Metallothionein has a protective effect against oxidative species damage. The aim of the present study is to investigate the effect of 8 weeks of aerobic activity on Troponin T levels and Metallothionein of cardiac tissue in healthy male rats.

Material & Methods: In this study, 20 Wistar male rats, aged 2 months with the weighted average of 180 ± 20 g, were selected and randomly divided into 2 groups: aerobic exercise (10) and control (10). The exercise group performed physical exercise 5 days a week according to the training program for 8 weeks. During this period, the control group did not exercise at all. After 8 weeks of training, independent t- test was used to analyze the data.

Results: The results demonstrated there was no significant difference between the average Troponin T (P=0.77) in the exercise and control groups. No significant difference was also observed between Metallothionein in the training and control groups (P=0.15).

Conclusions: It was revealed that the aerobic program used in the present study did not lead to increased cTnT which is indicative of cardiac injury. However, it increased Metallothionein levels (though insignificant) that can be helpful in preventing cardiac injury.

Key words: Aerobic Exercise, Troponin T, Metallothionein, Cardiac Tissue

1. Introduction

Despite improvements in healthcare, cardiovascular disease is still considered as the leading cause of death all over the world. Likewise, it is the leading cause of death in Iran. In developed countries such as the US, there were about 82.6 million Americans who were suffering from one or more than one type of cardiovascular disease in 2011 (1, 2). During the past 2 decades, cardiac troponin (CTNI) have been examined as biomarkers for non-invasive diagnosis of myocardial damage (3). Cardiac troponins are thin strands of sarcomere components that regulate the excitation-contraction coupling of skeletal muscles especially heart. Cardiac troponin consists of three subunits (C, I and T) of which only troponin T (cTnT) and troponin I (cTnI) are expressed to be specific isoforms of the cardiac muscle (almost exclusively) in thin Myofilament

in the contractile apparatus (4). Cardiac troponins are certain parts in myocytes which exist as large and small cytosolic free pools in sarcomeric structure (3-5%) (5). After membrane damage, both of these pools appear in the blood vessels. CTnT which are intended markers for myocardial necrosis diagnosis happen to be more important due to their superior sensitivity and specificity in the Cardiac tissue compared to cardiac enzymes or creatine Kinase-MB (CK-MB). Myocardial ischemia resulting in irreversible myocardial necrosis is known as the main pathomechanism for troponin release. Measuring Cardiac troponin is an almost whole algorithm structure and approved guide adopted in ACS diagnosis. In particular, troponin is the selective marker to diagnose myocardial infarction (MI) (5, 6). On the other hand, free radicals produced in body provoke potential biological damage which is called oxidative stress and nitrogen stress. Oxidative stress is created by metabolic reactions that need oxygen resulting in an imbalance between oxidant and antioxidant in vivo. Excessive ROS can damage lipids, proteins or cellular DNA which prevents their normal operation (7). Metallothioneins (MTs) are a family of small intracellular molecules (molecular weight ranging from 6000-7000 Daltons). These proteins are rich in cysteine. MT, as an antioxidant, plays a substantial role against damaging free radicals. MT has four main isoforms; the greatest expression of isoforms is in mammals, MT-1 and MT-2 (8). Metallothioneins has a key role in protecting organs and tissues against the toxic effects of heavy metals (9). Metallothioneins (MT-2) obtained from human cardiac cells have shown to protect the heart from oxidative species. Metallothionein is a metal-binding protein and cardio protective. In order to comprehend the molecular mechanisms underlying the role of MT in heart, in an experiment done in a published study, MT-2 stability was evaluated more than its cardiac cell expression and anti-oxidative properties. The analysis also revealed that the oxidative protection had increased significantly and it is beneficial for dissecting MT mechanisms in heart protection (10). The high prevalence of cardiovascular diseases is attributed to poor lifestyle including inactivity and lack of sports activities. Considering the high price of drug treatment, it is definitely more economical to change one's lifestyle in order to reduce the risk of factors associated with cardiovascular diseases. Therefore, exercise is a

logical approach to treat these diseases. Unlike other methods (such as medical and pathological procedures), it is safe, cheap and widely available (11). At the present time, one of the practical ways to protect the heart against myocardial damage is aerobic exercise. Regular aerobic exercise protects the heart from all levels of cardiac damage (12). On the other hand, it has been suggested that mechanical stretching, necrosis, and oxidative stress are the mechanisms of damage to the heart cells and the release of troponin through exercise (13).

Legazaris and colleagues (2015) examined the effect of triple exercise (three types of exercise including swimming, running and cycling) on cTnT levels in male athletes for 60 minutes and measured cTnT levels 5 minutes, 1, 3, 6, 12 and 24 hours after the exercise. The results illustrated increased levels of troponin in all participants (14). This kind of heavy physical training can increase the risk of cardiovascular events. Most referrals to the hospital with symptoms of cardiac troponin increase have been related to aerobic exercises such as marathon, cycling long ride or long distance walking (15). In another study, Chen and colleagues (2008) investigated the protective effects of exhaustive exercise on Metallothioneins in the heart of rats with infarction. In this study, rats were divided into 3 groups: control, swimming workout and swimming workout + zinc (Zn) consumption. The exercise program consisted of 8 weeks of swimming workout to exhaustion. The results showed that levels of Metallothioneins in the swimming-workout group decreased in comparison to the control group while Metallothionein levels increased in the swimming workout + zinc (Zn) group compared to the swimming-workout group (16).

Therefore, bearing in mind the contradictory results and the role of cTnT protein in the diagnosis of cardiovascular diseases, it has been widely regarded as a biomarker for the diagnosis of cardiac damage. Considering the role of Metallothionein protein in protecting the body against heavy metals and reactive oxygen species damage, regulating metabolism via Zn donation, separating and/or controlling oxidation / rehabilitation and limited number of researches done in this area, the emphasis on doing the current study seems to be logical. Accordingly, the overall goal of this study is to examine the effect of 8 weeks of

aerobic activity on Troponin T levels and Metallothionein of cardiac tissue in healthy male rats.

2. Material & Methods

This is an experimental study that is done via control and examination group procedure. 20 two month old Wistar male rats, weighing on average 180 ± 20 g, were selected and randomly divided into 2 groups: aerobic exercise (10) and control (10). Rats were kept in the Animal House of Faculty of Physical Education and Sport Sciences of Tehran University with a 22 ± 2 degree temperature, humidity of 50-60% and light-darkness cycle of 12-12. Rats were given 10 grams of food per 100 grams of body weight based on weigh-in once every 3days. Water was also given to them in special 500 ml bottles for laboratory animals. All animals in the experiment were used and treated according to Iran's Convention policy for the protection of vertebrate animals for experimental and scientific purposes; the protocol was adopted by the ethics committee of Endocrine Sciences Research of Shahid Beheshti Institute. After the weigh-in, the rats ran on the treadmill at a speed of 12 meters per minute for 2 weeks in order to become familiar with the treadmill. Then, they started exercising according to the training schedule of aerobic group, 5 sessions per week for 4 weeks. The overall running time for rats was about 42 minutes (6-minute warm-up, 50-60% VO2max), 30 minutes of aerobic exercise (70-75% VO2max) and 6-minute cool-down (50-60% VO2max) (Table 1). During this period, the control group did not do any exercise at all. In order to eliminate the acute effects of exercise and uncontrollable variables such as subjects' stress while performing the exercise program, rats were ethically anesthetized 24 hours after the last exercise session by intraperitoneal injection of a mixture of ketamine (30 to 50 milligrams per kilogram of body weight, intraperitoneal) and Xylazine (3 to 5 mg per kg of body weight, intraperitoneal). The cardiac muscle of the rat was removed from its chest, rinsed in physiological saline and weighed on a digital scale with 0.0001 gram resolution. Then, it was immediately frozen using liquid nitrogen for subsequent measurements and kept in the lab freezer at -70 °C in the Cellular and Molecular Endocrine Research Center of the Institute for Endocrinology and Metabolism of Shahid Beheshti

University. Troponin T was measured using ELISA kits for rats (Rat Cardiac Troponin T (cTnT)) made in Cusabio Biothech Company, China, with 3.12 pg sensitivity. By rat ELISA kits made in the same company with 0.039 ng/mg sensitivity, Metallothionein (Rat Metallothionein (MT)) was assessed via ELISA method and according to the manufacturer's instructions on Chinese Elisa Reader of HUISONG Company. In this study, descriptive statistics were used so as to calculate the mean and standard deviation. In order to determine significant differences in the levels of troponin T and Metallothionein in the exercise and control groups, independent t- test was utilized. After data collection, the analysis was done using SPSS 19; the significance level of the present survey was $P<0.05$.

Table 1. Aerobic exercise program

Steps of training	Warming up	Main body training	Cooling down
Time of training (min)	6	30	6
Intense of training (%VO_{max})	50 to 60	70 to 75	50 to 60

3. Results

At the end of the study, the results demonstrated that after eight weeks of aerobic exercise, there was no significant difference (P=0.77) between the experimental and control groups with respect to Troponin T levels; the average cardiac levels of troponin T changed from 22.65 picograms per milliliter in the control group to 23.59 pg/ml in the exercise group. Moreover, Metallothionein average levels in the control group changed from 1.70 to 1.90 ng/ml in the exercise group. No significant difference (P=0.15) was observed in Metallothionein levels between the experimental and control groups after eight weeks of aerobic exercise which is shown in the statistical information related to the two groups in Table 2 and Figures 1 and 2.

Table 2. Descriptive statistics Troponin T and Metallothionein

Variable	Group	Mean $\pm$ SD	sig
Troponin T (pg/ml)	Control	22.65 $\pm$ 5.32	0.77
	Exercise	23.59 $\pm$ 9.84	
Metallothionein (ng/ml)	Control	1.70 $\pm$ 0.21	0.15
	Exercise	1.90 $\pm$ 0.40	

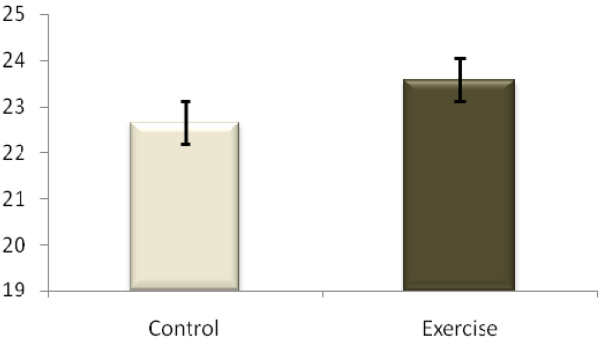

Figure 1. Mean ± SD of Troponin T in groups

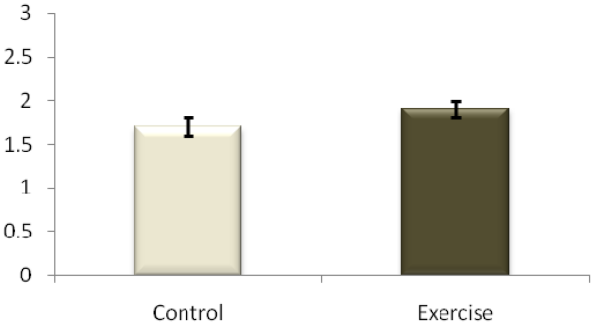

Figure 2. Mean ± SD of Metallothionein in groups

4. Discussion

The results showed there was no significant difference between the average troponin T of the exercise and control groups. No significant difference was perceived between Metallothionein of the exercise and control groups.

As the American College of Sports Medicine (ACSM) recommends, the best way to treat is to prevent by physical exercise. One of the best methods to prevent cardiovascular diseases is to do exercise. It has been proved that aerobic exercise improves cardiovascular function in human. Adaptations to aerobic exercise are related to increased maximal oxygen uptake, increased cardiac output and finally increased size of the left ventricle mass (17). Some studies have shown that performing long-term or intense exercise can evoke a stress response and stimulate significant

pathological changes such as apoptosis in skeletal muscles (skeletal and heart), liver and kidney. Although the mechanisms of cell death in various organs during exercise and after it are not fully understood, experts believe that some factors such as the kind of training program and ROS can be involved in this mechanism (18).

In a research study, Leetmaa et al. (2008) examined the cardiac function of 14 male triathlon after an official match, and troponin values increased significantly immediately after the competition but decreased at intervals 12-24 after the match (19). Recent research results with the results of the present study is not in a direction. The type of physical exercise can be mentioned as one of the important factors in both studies; aerobic exercise was performed in both studies. The duration of exercise is another important factor. It can be inferred that physical exercise within a specified time not only develop adaptation, but also does not lead to damage or the production of damaging factors.

Studies have shown that other physical activities such as exhaustive or strength and high intensity interval exercises can lead to tissue damage, which result in the release of cellular factors. CTnT evaluation is preferred to that of cardiac muscle enzymes in order to detect heart muscle damage in terms of sensitivity and specificity. Nowadays, elevated level of cardiac troponins is accepted as a standard biochemical index for the diagnosis of myocardial injury or infarction of short-term myocardium (20). Therefore, as shown in the present study, cTnT did not significantly increase with this type of aerobic exercise indicating that the exercise protocols of the study do not lead to cardiac damage. In addition, Völkers et al. (2013) illustrated that troponin T increased after doing exercise in Pulmonary Hypertension patients. Participants in this study included 24 patients who carried out sports medicine test (Bruce Test) (21). High blood pressure negatively affects myocardial structure and function, resulting in induced pathological concentric hypertrophy. It seems that cardiac hypertrophy response to excessive pressure is an attempt to normalize ventricular wall serving to maintain heart function in the face of an increased hemodynamic load (22). This increased hemodynamic load generates excessive pressure on the heart muscle of these patients which is eventually able to increase cardiac troponins.

Troponins are parts of myocytes existing as large and small cytosolic free pools in sarcomeric structure (3-5%). Both of these pools appear in blood vessels following membrane damage. cTnT, which function as expected markers for the detection of myocardial necrosis, are more important than cardiac enzymes or creatine Kynaz- MB (CK-MB) as a result of their superior sensitivity and specificity in the cardiac tissue (5). Unlike the study conducted by Welkers et al. the present study has not led to increased cTnT which might be due to differences in the subjects (patient and healthy), duration, intensity and the kind of exercise. High-intensity exercises can cause cardiac damage which should be prescribed with caution for people who suffer from cardiac disease. Thus, to do and prescribe exercise to patients, first and foremost, four main variables should be taken into account: frequency, intensity, time (duration) and the kind of physical activity. It is also necessary to consider the subject's physical conditions such as level of fitness and medical condition before prescribing an exercise. Joulazade et al. (2012) examined the effect of a progressive aerobic exercise program on MT levels of cardiac tissue in Wistar rats. The mice performed a progressive training program for 25 to 64 minutes at a speed of 15 to 22 m/min five times a week for eight weeks (23). In this study, MT was identified via Radio-immunohistochemical assay of cardiac tissue. The results showed that there was no significant change in MT resting levels of the training group compared to the control group after eight weeks of endurance exercise. The present study is in line with the research done by Joulazade et al. as it has not led to significant increase in MT. However, it has been confirmed that aerobic exercise leads to decreased levels of MT in cardiac tissue due to body adaptation against reactive species damage. Aerobic exercise makes the body adapt to the increased production of damaging substances particularly reactive oxygen species. This adaptation results in reduction of increased factors such as Metallothioneins that opposes these species. So, it is logical that, in this study, MT has not significantly increased while based on the data, Metallothionein can be introduced as an antioxidant factor to prevent damage against these factors. Exercise can have positive or negative effects on biological oxidation/reduction depending on the type (acute or chronic), characteristics and the duration of the exercise (24). Beneficial changes have been observed in

several physiological and biochemical parameters in general as a result of regular exercise of moderate intensity. In contrast, acute exercise such as interval training with high intensity may improperly and reversely increase oxidative stress and induce adverse effects on one's health. It is almost difficult to estimate the state of oxidative stress due to the complexity of the oxidant system / antioxidant defense and their delicate balance (25).

5. Conclusion

Finally, in spite of the health benefits of physical activity and reduced risk of diseases, it has been found that there are also some risks associated with training programs. Physical exercise increases metabolic requirements of the heart and biological factors, enhancing cardiac function with regard to the type of exercise. This increase can intensify the risk of heart attack (especially in patients). Consequently, duration, intensity, time and type of training program should be carefully investigated before starting the exercise and participating in training programs. The aerobic program of this study illustrated that it did not lead to increased cTnT which is one of the indicative factors of cardiac damage. Furthermore, it produced increased levels of Metallothionein (though insignificant), which can prevent cardiac damage. It seems that the training program of the present study can be prescribed for different people (sick or healthy).

Conflict of interests: No conflict of interests amongst authors.

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