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ORIGINAL ARTICLE

Response of MIR-1 and HSP-60 Gene Expression to Endurance Training in Heart Tissue of Rats

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KEYWORDS

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ABSTRACT: MicroRNAs and heat shock proteins are important factors in heart function. However, the response of these factors to exercise in the heart tissue is unclear. Here, we evaluated the impact of endurance training on the expression of MIR-1 and HSP-60 genes in heart tissue of rats. In this study, 10 male Wistar rats were randomly divided into 2 groups control and endurance training. The aerobic exercise program included running on the treadmill at speed of 25 m min^{-1}, 5 days a week for 12 weeks. After anesthesia, we performed an autopsy to collect the heart. The expression level of MIR-1 and HSP60 were measured by Real-Time PCR. An Independent t-test was used to determine significant changes ($P<0.05$). After the intervention period, the expression level of the MIR-1 gene showed a significant decrease in the aerobic exercise group thank in the control group ($P=0.001$). However, aerobic training had no significant effect on the expression level of HSP60 in the heart ($P<0.05$). It seems that twelve weeks of moderate-intensity aerobic exercise can probably improve heart function.

INTRODUCTION

Cardiovascular disease is the common cause of morbidity and mortality worldwide. Almost half of adults have at least one major risk factor for cardiovascular disease (ie, hypertension, high cholesterol, or smoking) [1, 2]. Cardiovascular disease encompasses a wide range of conditions including arrhythmia, hypertrophy or idiopathic cardiomyopathy, heart failure, and atherosclerosis that affects the cardiovascular system [3]. These conditions can potentially lead to fatal cardiac events including stroke, myocardial infarction (heart attack), or cardiac arrest. Therefore, the response of the involved factors and different therapeutic approaches are crucial to preventing or reducing cardiovascular disease [4].Evidence has shown that some miRNAs play an important role in the progression of heart disease and some also in the improvement of heart function [5, 6]. Similarly, MicroRNA-1 (MIR-1) expression in cardiomyocytes has been shown to reduce oxidative stress-induced apoptosis and to have protective effects against H_2O_2-induced cell death [7]. MIR-1 is the most abundant MIR involved in the development of the heart, which is involved in differentiation of cardiac precursors and their exit from the cell cycle. The role of miRNAs in the process of cell death after myocardial infarction has been demonstrated, as MIR-1 seems effective in preventing apoptosis. MIR-1 expression regulates cardiac cell the differentiation, ventricular growth, and conduction, respectively by regulation of HDAC4 (a factor that induces chromatin compaction), Hand2 (a transcription factor essential for cardiac growth), and Irx5 (cardiac repolarization regulator [8]. Studies have shown that miRNA-1 regulates several important factors including apoptosis by targeting heat shock protein 60 (HSP60) synthesis. HSPs modulate the proteins of the

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BCL-2 family, preserving the electrochemical gradient of the mitochondrial membrane and ultimately preventing the death of apoptotic myocardiocytes [9]. HSP60 can activate the innate immune system through the Toll-4 receptor and lead to cardiac myocyte apoptosis [10]. In addition, HSP60 is involved in myocardial injury during ischemia [11].

Recent studies showed physical activity is related to reduce inflammatory markers and decreased risk of heart failure and improved metabolic health [12-14]. The results are inconsistent with the effect of exercise on cardiac tissue mir-1 gene expression changes. It has been reported that miR-1 gene expression is highly responsive to exercise [15]. In a study aimed at investigating the effect of aerobic exercise on cardiac MicroRNA-1 at non-pathological levels in obese mice, it was shown that aerobic exercise reduced cardiac micro-RNA-1 to non-pathological levels [16]. However, the results of a study examining the effect of 14 weeks of resistance activity on a treadmill showed that resistance activity had no significant effect on cardiac miR-1 expression [17]. There was also a decrease [18] and no significant change [19] in HSP60 gene expression following exercise.

While the prevention and treatment of cardiovascular disease with minimally invasive catheter-based approaches have improved dramatically in recent years, there is a growing focus on non-pharmacological health strategies such as physical activity to maintain cardiovascular health. It is undoubtedly more effective than the treatment of cardiovascular disease clinically. The positive impact of exercise on cardiovascular health and the reduction in mortality of cardiovascular disorders have been well documented [14]. However, findings regarding the effectiveness of aerobic training on cardiac performance indices are limited. Since a few studies investigated the effect of exercise on the expression level of factors affecting cardiac function indices and the results were inconsistent, this study aimed to determine the response of MIR-1 and HSP-60 gene expression to endurance training in heart tissue of rats.

MATERIALS AND METHODS

In this study, 10 male four to six weeks old Wistar rats were selected from the Pasteur Amol Institute and transferred to the research center. Rats were randomly divided into control and training groups (each with 5 rats). Samples during one week of laboratory environment familiarization with the Mazandaran University of Physical Education and Sport Sciences and treadmill and protocol implementation in groups of five mice were maintained in transparent polycarbonate cages, at 20°C. Up to 24°C, 45-55% humidity, and dark to brightness cycle at 12: 12 h. Sterile wood chips and cuttings were used to absorb the urine and feces of the samples. Cages were washed and wood chips were replaced every day. In this study, the food of the samples was produced by Behparvar Amol Company, which was placed daily in the cage. The water required for the samples was freely dispensed in a 500 ml bottle for laboratory specimens. The aerobic exercise program included running on a treadmill at a speed of 25 m min^{-1}, five days a week for 12 weeks [20] (Table 1).

Table 1. Exercise training protocol.

Training sessions	**Weeks** **Training factors**	**First week**	**Second week**	**Third week**	**Fourth week**	**Fifth to twelfth**
First	Speed (m min^{-1})	10	15	15	20	25
	Duration (minutes)	15	20	35	50	60
Second	Speed (m min^{-1})	10	15	20	25	25
	Duration (minutes)	15	25	40	55	60
Third	Speed (m min^{-1})	10	15	20	25	25
	Duration (minutes)	15	25	45	55	60
Fourth	Speed (m min^{-1})	10	15	20	25	25
	Duration (minutes)	20	30	45	55	60
Fifth	Speed (m min^{-1})	15	15	20	25	25
	Duration (minutes)	20	35	50	60	60

In each group, rats were anesthetized by intraperitoneal injection of ketamine (50 kg mg^{-1}) and xylazine (3 kg mg^{-1}). The heart tissue was collected, following washing in physiological serum, and it was immersed in 1.8 microtubules containing RNAlaterTM fluid (a ratio of 20%) to transfer to the laboratory. Gene expression levels of the factors of interest were quantified by Real time-PCR technique and analyzed using the formula $2^{\Delta\Delta ct-}$. The PCR was performed using PCR master mix (Applied Biosystems) and SYBR Green in ABI Step One (Applied Biosystems, Sequence Detection Systems. Foster City, CA) according to the manufacturer's protocol. The normal distribution of variables was checked by the Shapiro-Wilk test, followed by an independent t-test at the $p \leq 0.05$ to examine the differences between the groups.

RESULTS

Table 2 demonstrates the mean and standard deviation of gene expression levels in different groups. The effect of 12 weeks of training on the expression level of the mir-1 gene was determined using an independent t-test at a significance level ($P \leq 0.05$). The results showed that endurance training significantly reduced the expression level of mir-1 compared to the control group ($P<0.001$) (Figure 1).

However, the expression level of HSP60 in the rat hearts in the training group showed no significant difference compared to the control group ($P<0.05$) (Figure 2).

Table 2. Mean and standard deviation of genes expression levels in control and aerobic training groups.

Variables **Groups**	**MIR-1**	**HSP-60**
Control	1.89±0.83	1.06±0.42
Aerobic training	1.01±0.19	0.7±0.17

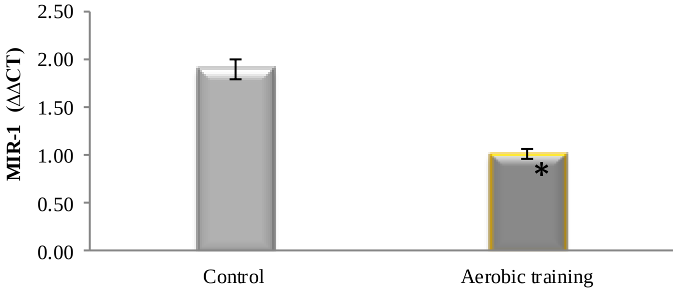

Figure 1. The expression level of MIR-1 in the rat hearts in the control and experimental groups
*Significant difference compared to the control group ($P \leq 0.05$)

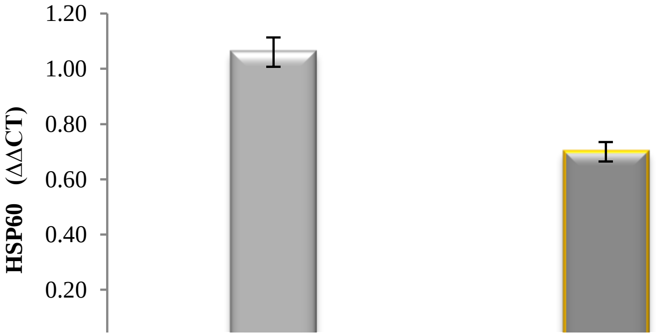

Figure 2. HSP-60 gene expression changes in rat heart tissue in control and experimental groups.

DISCUSSION

In this study, we showed that endurance training intervention led to a significant reduction in the expression level of mir-1 in the heart tissue of rats. This finding was consistent with the results of another study on rats that showed mir-1 levels in heart muscle decreased with both types of moderate to high-intensity endurance training protocols [21]. Regarding the mechanisms involved in alterations of mir-1 gene expression in exercise training, some findings suggest that calcium and caspase-9 increase mir-1 gene expression. Increased calcium levels increase normal myocardial SERCA2 levels. It has also been suggested that cardiovascular myofibrils' susceptibility to calcium increases as a result of aerobic exercise and as result; calcium-dependent kinases such as calmodulin are activated [22]. In this connection, it has been shown that calcium-mediated signaling acts as the most important upstream mediator of cardiac hypertrophy. Interestingly, miR-1 regulates two distinct mediators of calcium signaling, including calmodulin activation (if mir-1 increases) and Mef2a (if MIR-1 decreases), which transcription of the cardiac calm1 and Calm2 genes, both have been shown in animal models of heart failure. Thus, downstream transcriptional effects, MEF2A and GATA4, are regulated by mir-1, indicating that miR-1 simultaneously plays an important role in regulating the expression of calcium signaling mediators [23]. Overall, in our study, given the increase in mir-1 after a period of aerobic training, consistent previous evidence may suggest that activation of Mitogen-Activated Protein Kinases (MAPK) such as PGC-1 may be activated. And calmodulin activation that activates HDAC (activation of HDAC-4 suppresses MEF2) and modifies the expression of the mir-1 gene by inhibiting caspase-9 and HSP60 / 70 and may eventually reduce heart damage [22]. However, the results of the present study are inconsistent with the findings of some studies. In a study, 14 male Wistar rats performed 14 weeks of endurance training on a treadmill. The results showed no significant difference in the expression of the 1-miR gene in the control and experimental groups [17]. The inconsistency with the above findings can be attributed to factors such as training protocol and the number of training sessions per week as well as sampling time. On the other hand, the inconsistency observed in different studies may return to the intensity of activity or type of activity [15]. A study on rats found that after compressive overload (a pathological inducer of hypertrophy), the decrease in the mir-1 is one of the fastest changes that occur even before the heart mass is increased [24]. On the other hand, mir-1 expression is different in heart failure. As some have reported, mir-1 expression is reduced in cardiomyopathy [25]. But others have expressed an increase. About this contradiction, has been reported that mir-1 decreases in hypertrophy but reverts to the first levels or more when the failure progresses [26]. Thus, the expression of mir-1 is associated with hypertrophy. However, as the physiological type of hypertrophy improves cardiac function and its pathological type decreases cardiac efficiency, there may be differences in the type of hypertrophy and the expression of mir-1 [27].

Another finding of the present study was a non-significant decrease in HSP60 levels in the training group. HSPs are known as stress proteins and play an important defense role in cardiovascular and muscle cell damage. HSPs can exert anti-inflammatory activity by inhibiting the production of inflammatory cytokines such as TNF-α by inhibiting the NF-Kb pathway at the cellular level [28]. Few studies have investigated the effect of exercise on changes in these elements in the heart tissue. The results of this study are consistent with the findings of some previous studies. In a study, 30 male Wistar rats were studied. The training was done on a treadmill for one month and 3 sessions per week. The results showed a decrease in HSP60 gene expression in the experimental group after ischemia-reperfusion but this decrease was not significant [19]. Positive changes in this factor indicate a protective effect that results in a significant protective effect against hyperthermia, increased tissue or serum TNF-α level, and may ultimately increase tissue resistance to ROS injury [29]. miRNA-1 regulates several important factors, including apoptosis, by targeting the synthesis of HSP60 and Bcl-2. HSPs also modulate the proteins of the BCL-2 family, preserving the electrochemical gradient of the mitochondrial membrane and ultimately preventing the death of aphotic myocardiocytes [30], Bcl-2 prevents oxidative cell destruction and is one of the most important apoptosis inhibitor proteins that binds to apoptosis protease activator (of-1) that in addition to preventing cytochrome c release from mitochondria, by removing H ions, it binds to the apoptotic protease activator (of-1) and inhibits caspase-9 activation [31]. Studies have shown that mir-1 correlates with apoptosis-related genes such as HSP and it has been reported that ischemia-reperfusion of cardiac cells has been associated with changes in mir-1 expression and HSP protein expression. Therefore, ischemia-reperfusion protects cardiac cells against ischemia-reperfusion injury not only by increasing HSP but also by increasing miR-1 [32]. Also, reported that mir-1 protects the heart tissue by enhancing eNOS stimulation, heat shock transcription factor-1 (HSF-1), and HSP [32]. Contrary to the findings of the present study, a study investigated the effect of combined physical activity (aerobic and resistance) on HSP60 expression and adipose tissue inflammation in diabetic subjects. 3 months of physical activity affected HSP60 expression and heat shock response differently. HSP60 expression was significantly increased in adipose tissue following exercise in the diabetic group, whereas it decreased in the non-diabetic group [18]. The inconsistency with the above findings can be related to factors such as the type of exercise and the type of subjects. Aerobic exercise was one of the strengths of the present study, as this type of exercise, despite its limitations, may have different responses and adaptations to other training programs. There were also limitations in this study, such as a lack of measurement of inflammatory cytokines in heart tissue. Measurement of calcium signaling pathways can also shed light on the effects of physical activity on transcription factors involved in cardiac muscle function. This is the weakness of the proposed research for future studies to measure these factors in the heart tissue. However, further research is needed.

CONCLUSIONS

In summary, the results of this study showed that twelve weeks of aerobic exercise regulated the expression of genes involved in cardiac function mechanisms. According to research findings, twelve weeks of moderate-intensity aerobic exercise can probably improve heart function.

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ETHICAL CONSIDERATION

The research was carried out at the Islamic Azad University, Ayatollah Amoli Branch, with the approval of the Ethics Committee under the number IR.IAU.M.REC.1399.015.

Conflict of interests

No conflict.

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