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Effect of Six Weeks Light Intensity Interval Training on ANP and BNP Gene Expression Levels after Myocardial Infarction

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Abstract

Introduction: Myocardial infarction (MI) is the irreversible cell death caused by ischemia in parts of myocardium**.** Atrial and brain natriuretic peptides (ANP and BNP) are known as strong markers of myocardial infarction. This research aimed to evaluate the effect of precedent of six-week light intensity interval training (LIIT) on the ANP and BNP gene expression in rats after Myocardial Infarction.

Method: Twelve Wistar male rats of 10 weeks old and mean weight 250-300gr were allocated in two groups with six Rats in each groups of 1. LIIT (60 minutes interval running on a treadmill, each cycle including four minutes of running with an intensity of 55-60% VO_{2max} and two minutes of active recovery with a 45-50% VO_{2max} intensity, three days a week for six weeks) and 2. control group (without any precedent of training). Real-time PCR was used to assess the expression of ANP and BNP genes in myocardium (after inducing MI By blocking left coronary artery by surgery). For statistical analysis of data independent sample t test ($p \leq 0.05$) was used.

Results: Light intensity interval training has significant effect on decreasing the gene expression of ANP ($p=0.01$) and BNP ($p=0.001$) in rats after myocardial infarction.

Conclusion: It appears that adaptation to light intensity interval training controls and moderates the secretion of cardiac hormones in rats after myocardial infarction.

Keywords: Myocardial Infarction, ANP, BNP, Light Intensity Interval Training

Introduction

Myocardial infarction (MI) is the destruction and permanent irreversible cell death of a myocardium's part which occurs as a result of the obstruction of myocardium's nourishing vessels and is often associated with symptoms such as increased enzymes (troponin T, creatine kinase, etc.) and cardiac hormones, the atrioventricular and peritonitis natriuretic peptides (atrial and brain natriuretic peptides or ANP and BNP, etc). Therefore, measuring the values of these hormones can have a diagnostic value in heart failure (1). Meanwhile, the ANP and BNP cardiac hormones are more important due to having many physiological effects including affecting kidney by increasing urinary volume and electrolytes, increasing sodium excretion as a result of their natriuretic properties, increasing the glomerulone filtration and decreasing the outflow and coronary artery abnormalities in the heart and expanding the lungs and affecting the central nervous system by preventing sympathetic activity, increasing lipolysis, regulating body temperature, increasing heart rate and decreasing blood volume and pressure and other hormones by preventing the secretion of arginine and vasopressin, reducing plasma renin activity, inhibiting the activity of catecholamines,

reducing aldosterone and cortisol, increasing testosterone levels, preventing the secretion of pancreatic intestine, disrupting insulin secretion, and metabolism and vascular smooth muscle, and therefore lowering blood pressure (1). ANP is a 28-amino acid peptide hormone which is released from the atrium myositis when it is stretched out (1). After being inserted into the plasma, ANP is bound to the ANP receptors (types A and B) in the brain and blood vessels as well as the kidneys and adrenal glands. ANP's connection to these receptors enables the activation of Guanillacyclaz (cGMP). The cGMP activates protein kinase, which in turn increases the activity of the calcium pump in the muscle's smooth cells. The result is a reduction in intracellular calcium that will smooth the muscle relaxation (2). The hormone also inhibits collagen synthesis in cardiac fibroblasts through the cGMP secondary messenger control and can damage the heart tissue (2). MI-induced fibrosis promotes the secretion of ANP by increasing transforming growth factor beta (TGFβ1), fibroblast growth factor and angiotensin II as well as macrophages (1, 2). BNP is also a neurohormonone made by the heart's ventricles and is released from myocytes in response to tension stress and volume overload as well as increased pressure. As a result of the pressures, pre-pro-BNP is converted to pro-BNP, and then to BNP (3). The BNP precursor is a 108-amino acids NT Pro BNP protein which, after being released by a protease series, breaks down to two BNP C Terminal Pro BNP molecules with 1 to 76 amino acids. The plasma half-life of NT Pro BNP and its plasma concentration is higher than that of BNP, so measuring plasma levels of this agent cannot accurately represent the levels of BNP secretion (3). Increasing the production and secretion of BNP is the result of increased levels of creatine and troponin in the heart. Due to the stretching of myocytes, troponin and creatine kinase enzymes increase and induce BNP release (4). There is also a significant correlation between the increase of BNP values and the left ventricular injection fraction (LVEF) (5). BNP levels increase in cardiac patients and heart muscle necrosis, which is a symptom of left ventricular dysfunction and is higher in patients with ventricular dysfunction. An increase in BNP levels indicates a deterioration in the status of coronary artery disease, and its value, such as the ANP, which can have a diagnostic value in heart failure (6). Lower rate of production and secretion of these two hormones, in addition to showing less damage to the heart tissue due to myocardial infarction, will also reduce probable kidney diseases and their problems. Therefore, any factor that can reduce their secretion is of interest to researchers, including exercise physiologists, and helps minimize the damage caused by this disorder and even its relative treatment (2). The role of regular physical activity in health has been well documented, however, most people do not follow regular physical activity. Endurance training has been considered today as a strong stimulus for cardiovascular adaptation and health (7). Since myocardium's volume and its elasticity are among significant factors affecting the secretion of ANP and BNP (8), it is likely that increased heart rate in endurance athletes will also be effective in secretion of these hormones. The release of NT-proBNP plasma (ANP and BNP antagonist) is also affected by several factors, such as an increase in inflammatory cytokines levels of interleukin- 6 (IL-6) (9) and also angiotensin II (10), which leads to a decrease in cardiac function and myocyte damage (11). As angiotensin II decreases in response to training (12), it could be hoped that adjusting these factors in response to a light intensity interval training (LIIT) would eventually reduce the release of ANP and BNP. Few studies have been done concerning the effect of compatibility with LIIT and in particular the study of the effect of training history on reducing the complications of MI and reducing the severity of the disorder and some previous

studies have investigated the therapeutic effect of training on subjects after MI or cardiac hormones response to a training session (1). For example, Ravasi *et al.* examined the effect of a session of endurance and speed activity on 24-hour male athlete plasma ANP. They showed that a session of endurance and speed of activity would significantly increase plasma ANP (13). Khalighfard *et al.* also investigated the effect of endurance training on cardiac markers and exercise-induced immune response in elite male riders. The subjects drove a distance of 8,000 m in intensity from 75 to 80 % of maximum heart rate for 60 minutes. The results indicated a significant increase in NT-Pro BNP and an insignificant decrease in IL-6 (14). In another study, Nathalie et al reviewed and compared the effect of a session of endurance training and high intensity interval training on patients with MI and 14 healthy subjects. They reported that 30 minutes of endurance training (65% VO_{2peak}) and 10-minute HIIT (10 sets of one minute with 90 % VO_{2peak}) significantly increased cardiac troponin I (cTnI) and BNP plasma (1). Thijs *et al.* also reviewed and compared the effect of endurance and interval training on the levels of cTnI and BNP in MI patients. Subjects were divided into two experimental groups including endurance training (30 minutes running with 65 % VO_{2peak}) and experimental group of high intensity interval training (10 sets of one minute with 90 % VO_{2peak}) and a healthy control group with mean age of 60 ± 6 years. The results showed that CTnT and BNP levels were significantly lower in the control group than the experimental groups and there was no difference between the two trainings (15). Guazzi *et al.* also examined the effect of 24 weeks of increasing aerobic training (40 minutes each session with 60 % maximum heart rate in the first two weeks and then gradually up to 80 % of maximum heart rate) on the levels of NT-pro-BNP in 26 MI patients (9.5 ± 67 years) in two control and experimental groups. The results showed a significant decrease in NT-pro-BNP in the experimental group compared to the control group (16). Regarding the results of previous studies, the role of training on cardiovascular health and its better performance before and after MI and during the period of remission after the onset of MI is indisputable. So the present study aimed to review the effect of six weeks light intensity interval training on ANP and BNP gene expression levels after myocardial infarction in rats.

Methods

In this fundamental research, the effect of six weeks of LIIT on cardiac hormones in Wistar rats after MI was evaluated. A total of 12 Wistar male rats (12 weeks old) were purchased as a statistical sample from the Razi Vaccine Institute. They were kept in separate cages with free access to water and food packs based on NIH-publication in a 12-hour sleep and wake cycle. At first, the rats started with a gentle walk on treadmill at a speed of five meters per minute, five minutes a day and four days a week (17). Rats' VO_{2max} were measured by maximum exercise activity, according to the formulas and table set out in Morten *et al.* (2007), Wisloff *et al.* (2000), to estimate the initial speed of rats' running (17, 18). To measure VO_{2max}, the rats were first warmed for 10 minutes, and then the VO_{2max} test started at 0.1 M/m and the treadmill speed was increased every 0.1 M/m as long as the rats were completely exhausted (in the case of exhaustion, the rats lost control and did not respond to the treadmill automatically) and the exhaustion rate of MI-rats was converted to VO_{2max} by the formula y = 1.14x + 9 (17, 18).

x = speed of exhaustion with unit m / min.
VO_{2max} = y in ml units per kilogram of body weight per minute.

Each rat's speed on the treadmill was calculated individually according to the maximum oxygen consumed by it. Finally, the rats were randomly divided into two groups, LIIT and control group and six weeks of LIIT protocol was conducted in the LIIT group. In

the LIIT group, rats warmed up five minutes before the start of the main training session. The rat's speed gradually increased by 0.2 M/m per week as an overload, according to the formula of Kraljevic *et al.* (19), and the treadmill slope throughout the entire training period was zero (17, 18). In contrast, control group rats did not perform any training. The LIIT protocol included initial warm-up before training for five minutes at a speed of five meters per minute and then a 60-minute interval running on the treadmill. Each cycle consisted of four minutes of running with intensity of 55- 60 % VO_{2max} and two minutes of active recovery with a 45- 50 % VO_{2max} intensity, three alternate days a week for six weeks (19). According to the formula of Kraljevic *et al.* (19), the rats' speed was gradually increased with 0.02 M/m per week as an overload and the treadmill slope was zero degrees throughout the training period (17, 18). It should be noted that control group rats did not training during six weeks. After six weeks of training, both groups (experimental and control) underwent surgery and their left coronary artery bypass (LAD) was blocked, and the rats were infected with MI. To ensure that the rats were infected with MI, all of them were deplored with echocardiography (branded by Jay Halshore, USA). During this process, the Left Ventricular Diastolic Dysfunction (LVDd), left ventricular diameters at the end of the systolic (LVDs), End-diastolic volume (EDV) and End-systolic volume (ESV) were examined. The ejection fraction of the left ventricular (FS) and left ventricular drainage fraction (FE) were calculated relative to the formulas (19):

EF = (LVDd2-LVDs2) / LVDd2
FS = ((LVDd - LVDs) / LVDd) * 100

Rats with a FS≤35% were selected for this study as MI rats (19) and heart muscle tissue samples of left ventricle were taken to measure the RNA levels of ANP and BNP genes. Samples were transferred to the genetic laboratory after freezing and measured by Quantitative Real Time PCR (qRT-PCR) and the following steps were taken: (1) preparation of ANP and BNP samples, (2) RNA extraction of samples, (3) investigation of optical absorption of samples by spectrophotometer, (4) RNA DNA synthesis, (5) performing a real-time PCR reaction, (6) evaluation of ANP and BNP gene expression in LIIT and control groups (by laboratory kit with korean brand Bioner and Rayleigh Time Tester PCR with brand SteppenAbI in America and primer with brand Matter in Germany). After performing the reaction, the ΔΔct method was used for quantitative analysis of Real Time PCR data. The ct data was extracted from the machine and then, using the Graph pad software, the gene expression was plotted. Quantitative data were analyzed by Real Time PCR using SPSS18 software.

The primer sequence of the ANP was:

"ANP forward: 5'-CCCAATCCACTCTGGGCT-3"
"ANP reverse: 5'-TTTGGAGGACAAGATGCCT-3"

The sequence of BNP was:

"BNP forward: 5'-TTGCAGCCCAGGCCACTGA -3"
"BNP reverse: 5'-AGCTGTTGGACCGTCTACGA-3"

The collected data were analyzed using the SPSS18 software. Kolmogorov-Smirnov test was used to determine the normality of the data. Given the normal distribution of data, independent t-test was used to analyze the data ($p \leq 0.05$).

Results

Mean and standard deviation of ANP and BNP gene expression levels in LIIT and control groups are presented in Table 1. The results of independent t- test in Table 1 showed that ANP gene expression levels in LIIT group was significantly lower than the control group (p=0.01), Also the results of independent t-test in Table 1 showed that BNP gene

Table 1. the results of independent t- test for compare the ANP and BNP gene expression levels in LIIT and control groups

Variable	Group	Number	Mean ± Standard Deviation	t	p
ANP	LIIT	6	1.009±0.152	3.93	0.01
	Control	6	4.333±2.062		
BNP	LIIT	6	1.002±0.083	10.40	0.001
	Control	6	8.927±1.863		

expression levels in LIIT group was significantly lower than control group (p=0.001).

Discussion

The results of this study showed that six weeks of LIIT significantly decreased ANP and BNP gene expression. The results of this study were consistent with Guazzi *et al.* (2012), which examined the effect of a LIIT and reported an increase in NT-proBNP But it was inconsistent with the results of research by Thijz (2015) and Nathalie *et al.* (2015). The contradiction in results can be due to the difference in training protocol and duration of training, as well as the time to measure the factors. In these studies, the effect of a training period was not investigated and the changes of these factors were studied after a session of activity. Also, in previous studies, the effect of LIIT after MI was investigated. The health of subjects in some previous studies may also be another factor in the inconsistency of their results with the present study. In most of the previous studies, the plasma levels of NT-proBNP have been studied that Due to its half-life and its higher concentration, the BNP ratio cannot be as accurate as the analysis of ANP and BNP gene expressions. Since NT-proBNP is known as a common antagonist of ANP and BNP, and the cellular signaling pathway has produced and secreted the same two hormones, they have been simultaneously studied. It seems that in the present study, the concentration of ANP and BNP stress hormones has been moderated by adaptation with LIIT. These physiological adaptations have caused the sympathetic system and stress hormones to be involved, allowing for complete structural and physiological recovery and increased cardiac function (20). Although evidence suggests that a training session of any intensity will increase cardiac hormones (12), but adaptation to LIIT (as the periods of rest between periods of activity reduces training pressure and allows longer-term activity for people of all ages with any physical condition, and will also benefit from the benefits of recovery from the recovery period) can control and modulate the secretion of cardiac hormones in training as compared to those without a history of training by affecting the secretion and function of chemical pixels (10). Due to the adaptation with LIIT, nitric oxide is moderated, the Gene Cards Protein (GC) is activated and Modifies the amount of circular guanosine monophosphate which causes less guanosine monophosphate production. As a result, the phosphordiesterase enzyme (PDE) activity is lowered and fewer calcium channel closes. As a result, the cAMP that increases cardiac contractility less degrades the phosphordiesterase to the inactive Adenosine-5-monophosphate (AMP-5) nucleotide, resulting in less protein kinase G (PKG) enzyme activation (10,20). Probably, six weeks of LIIT has also been able to modulate the plasma NT-pro-BNP by modulating the secretion of inflammatory cytokines (21). LIIT may also affect the function of the ANP-NPR-A system by changing the number and activity of receptors of the natriuretic peptides (9). On the other hand, adjustment with a six-week LIIT may reduce the ventricular wall pressure during diastole and ultimately reduce NT-pro-BNP (22). Another factor likely to be effective in reducing ANP and BNP following

adjustment for six weeks of LIIT is increasing the number of hormone receptors, which in turn leads to an increase in the binding of BNP and ANP, and ultimately to a reduction in blood pressure (9). In general, the results of this study support the role of LIIT on reducing the expression of ANP and BNP genes after MI. In this study, the effect of six weeks of LIIT was studied. Therefore, it is suggested that researchers in future studies investigate the effect of other training intensities and compare them with each other.

Conclusion

According to findings of present study it can be concluded that adaptation to light intensity interval training controls and moderates the secretion of cardiac hormones in rats after myocardial infarction.

Ethical issues

No applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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