Interval Training Intensity and the Expression of Caspase-9 in Obese Rats with Myocardial Infarction

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Received: 08 April 2017

Accepted: 04 June 2017

Published in July 2017

Abstract

Objective: Physical exercise reduces myocardial apoptosis but its molecular process is unclear yet. The activated Caspase-9 has a key role in advancing the process of apoptosis. The aim of this study was to evaluate the effect of aerobic interval training intensity on the process of apoptosis in the 10-week male obese wistar rats with myocardial infarction.

Materials and Methods: In this clinical trial,18 rats after myocardial infarction induction were divided into three experimental groups of high intensity (Interval running for 60 minutes on a treadmill, each cycle consisting of 4 minutes running with 55-60% of VO2max and 2 minutes' active recovery with an intensity of 45-50% VO2max, four days a week for 6 weeks) and low intensity (Interval running for 60 minutes on a treadmill, each cycle consisting of 4 minutes running with 85-90% of VO2max and 2 minutes' active recovery with an intensity of 50-60% VO2max, four days a week for 6 weeks) and a control group (no training). The concentration of cardiomyocytes caspase-9, as the main indicator of apoptosis was measured by qRT-PCR.

Results: The concentration of caspase-9 was higher in the control group than the high intensity training group (P=0.012) and the low intensity training group (P=0.002). The high intensity training group had higher Caspase index values than the low intensity training group ($P\leq$0.001).

Discussion: The findings of this study indicated aerobic interval training resulted in the expression of caspase-9 gene and thus reducing the occurrence of cardiomyocytes apoptosis after myocardial infarction.

Keywords: Apoptosis, Interval training intensity, Myocardial infarction.

Introduction

Myocardial infarction (MI) is the permanent and irreversible cell death of a myocardium part which occurs due to impaired blood flow and the occurrence of intense myocardial ischemia as a result of coronary artery occlusion (1).

Tissue damage caused by MI is the outcome of primary ischemia, especially influenced by the duration of ischemia and is a result of myocardial reperfusion. Although oxygen values return to its first level by reperfusion, respiratory chain compounds are in the reduction mode and a wave of reactive oxygen species is particularly seen at the beginning of reperfusion. Together, these events cause mitochondrial permeability pores resulting in cell death through apoptosis (2).

Apoptosis or programmed death of a cell is a physiological and biological process for active and normal growing as well as maintaining homeostasis. Programmed cell death is triggered via different routes (3). In this process Caspase which increases under the influence of apoptosis starter proteins called BAX have the main role (3). The need for continuing apoptosis depends on whether the cell damage is beyond the ability of the cell to repair itself or not? This is also dependent on the ratio of promoter apoptosis proteins (Fas and p53) and suppressor proteins (Bcl-2) (4).

Caspases are cysteine proteins that separate terminal aspartic acid residues (ASP) (such as cysteine Spartaz) and are inactively present in the cytoplasm to all cells (5,6). To date, 14 caspases which are involved in cell death have been identified. Caspases decay the precursors of the cytokine growth (3). Apoptotic death messages are announced by caspase-8 and caspases-3, 6, 7. Caspase 9 starts apoptotic programs to decompose a number of vital proteins. In general, increased levels of caspase increases apoptosis and decreasing caspase leads to cell survival and repairing (4). This balance changes in physiological and pathological situations and regular exercise is one of those situations. Physical activity may prevent cell death by influencing factors affecting the process of apoptosis. Also physical activity as an effective way to improve the quality of life of patients with myocardial infarction is known.

Today, endurance trainings are more noted as a significant stimulus for cardiovascular adaptations. Also, high intensity interval training with a minimum time is more noted to overcome the problem of opportunity to participate in physical activity and ultimately increasing physical activity and health (7).

Various studies have previously achieved contrary results about the effect of different intensities of physical training on the process of apoptosis. For example, Lu et al. compared the effects of HIIT and endurance training on symptoms of apoptosis and cardiac function in female rats. They assessed injection-fraction of the left ventricle as well as apoptosis markers including PI3K, AKT, AMPK as a result of mitochondria hurt and mitochondria function reduction. Results indicated the significant effect of HIIT and aerobics training on decreasing the process of apoptosis (8). Also, Hsp70 increases, ApaF-1 decreases and no significant change of caspase-3 after eight weeks of regular training with low intensity in myocardium and skeletal muscle of rats were reported (9). In another study, 10 weeks of swimming regular aerobic training inhibited apoptosis in male wistar rats (10). Interestingly, one session eccentric exercise increased caspase levels (11) which shows the effects of physical activity on the process of apoptosis. As mentioned, no study conducted regarding the effect of low intensity training and high intensity interval training on apoptosis process at myocardial infarction risk. These studies showed that apoptosis reply to training depends on the intensity and type of training. This study seeks to answer the questions that what is the effect of high intensity interval training and low intensity interval training on caspase-9 concentration and ultimately apoptosis in myocardial infarction?

Materials and Methods

Animals

In this experimental study, 18 male wistar rats were selected and randomly divided into three groups of 6 rats including high intensity interval training, low intensity interval training and control. The rats were kept in separate cages with free access to water and food

parcels according to the principles of Laboratory Animal Care (NIH-publication) in the 12-hour sleep-wake cycle.

Myocardial infarction induction

MI was induced in rats through direct intervention methods. In this method, the left anterior descending (LAD) coronary artery in rats was blocked by suture Silk 06. Rats were initially kept in a lab environment in the center of empirical research at Shahid Rajaii Hospital for one week. Then, rats were anesthetized by ketamine (150mg/kg) and xylazine (15mg/kg) and their chest hair were fully revised and put under medical ventilator (12). Then, a horizontal shear of 4 to 5 cm was created on the left side of their chest by the scalpel and other surgical tools so that myocardium is fully visible after pushing the chest. LAD is fully clear at this stage and then is completely blocked by suture. After LAD occlusion, chest, muscles and skin were sutured, respectively. The rats which had surgery remained under medical ventilator to become naturally to consciousness and began to breathe. Finally, the rats were placed in separate shelves to go through echocardiography after a week.

Preparation and training program

After MI, rats passed the recovery period for two weeks. In the third and fourth weeks, the rats were familiar with the treadmill by walking slowly (at a speed of 5 meters per minute for five minutes a day, 4 days a week). At the end of week 4, rats' VO2max was measured through maximal exercise test to estimate their initial running speed (13). Each rat's running speed on treadmill was calculated individually based on their VO2max. Rats rested for two days. Then, to ensure MI induction, rats were anesthetized for measuring hemodynamic parameters by echocardiography. During this process, left ventricular fraction shortening (FS) index was measured. For this study, rats having FS≥35 percent index were selected as rats with MI (12). Finally, the survived rats with myocardial infarction were randomly divided into three experimental groups of high intensity interval training (HIIT), low intensity interval training (LIIT) and control group (CTRL). The exercise protocol was implemented (12).

In HIIT experimental group, rats had interval running training four days a week, for 6 weeks, 60 minutes each session. Each cycle consisted of 4 minutes running with 85-90% VO2max and 2 minutes' active recovery with an intensity of 50-60% VO2max. A warm up period for 8 minutes at a speed of 5 meters per minute before starting the main training phase was done.

In the LIIT experimental group, rats had interval running training four days a week, for 6 weeks, 60 minutes each session. Each cycle consisted of 4 minutes running with 55-60% VO2max and 2 minutes' active recovery with an intensity of 45-50% VO2max (13). A warm up for 8 minutes at a speed of 5 meters per minute before starting the main training phase was done. It should be noted that rats in the control group had no training, but they were removed from the cage and put beside a ready treadmill to control the effects of running intention on a treadmill during the day.

Rats' biopsy

After the six weeks study period, rats rested for two days in order to avoid the last activity session. They were anesthetized for echocardiography again and myocardium's biopsies in MI area. So the amount of caspase-9 gene RNA was measured.

Stages of gene expression measurement

1. Caspase-9 sample preparation
2. RNA extraction of samples
3. Check the optical density of the samples with a spectrophotometer
4. The synthesis of cDNA from the RNA
5. Real time PCR reaction
6. Evaluation of caspase-9 gene expression in samples of the experimental group and the control group.

Statistical Analysis

The collected data were analyzed using the SPSS-18 statistical 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 and ANOVA was used to compare the three groups at a significance level of 0.05.

Results

The results showed that caspase index values in the control group was greater than the HIIT experimental group (P=0.012) and the LIIT experimental group (P=0.002). Also, caspase index values were higher in the HIIT experimental group than the LIIT experimental group (P≤0.001). Table 1 shows the comparison of caspase-9 in the study groups.

The results of the independent t-test showed, there was no significant difference in the caspase index of the two groups of HIIT and LIIT (P≤0.001). But a caspase-9 index value was higher in the HIIT experimental group than the LIIT experimental group.

Results of this study showed that the caspase-9 mean value was higher in the control group (8.07) than the HIIT experimental group (3.56) and the LIIT experimental group (0.95). Hence, training intensity has a significant effect on the enzyme concentration in a way that LIIT more reduces the enzymes concentrations (table 1). The caspase-9 mean value in different groups of the study is shown in figure 1.

FS echocardiography index

Table 2 shows the change amounts of EF and FS in one and ten weeks after surgery (including four weeks' recovery after surgery and six weeks of physical activity intervention). Also, Table 2 indicates the EF and FS values of the healthy control group (Control).

Discussion

Caspases are the member of cysteine proteases family and play a central role in apoptosis initiation and implementation phase. Evaluation of caspase activity is viewed as a biochemical marker of apoptosis.

The findings of this study suggested six weeks of HIIT and six weeks of LIIT effect on reducing the expression of caspase-9 as the most important factor influencing the process of apoptosis in rats with MI. Our findings are consistent with the results of Lu et al. (2015). It was one the comparative study on the effect of HIIT on myocardial apoptosis induced by mitochondrial dysfunction. Their study compared HIIT and endurance training on the symptoms of mitochondrial function, apoptosis and cardiac function in female rats. Also, our findings were in line with the results of Parco et el. (2004) which examined the effect of eight weeks of regular LIIT (5 days per week) in rats' cardiac and skeletal muscle. They reported increased Bcl-2, Hsp70 and reduced ApaF-1, Bax as well as no significant change in caspase. However, our findings were not consistent with the results of Biral et al. (2002) who declared that caspase values increase after one session of extrovert aerobic

Table 1. The comparison of caspase-9 in the study groups

Group	Control Mean ± standard deviation(SD)	HIIT experimental Mean ± SD	LIIT experimental Mean ± SD
Caspase 9 (mg/ml)	8.07±2.95	3.56±0.76	0.949±0.002

Table 2. Changes in Ejection fraction and Fraction shortening (mean ± SD) in the study groups

Variable and Group	Echocardiography time	Injection fraction (%)	Fraction shortening (%)
LIIT	One week after surgery	56.862±9.926	25.890±5.789
	Ten week after surgery	72.872±8.951	37.724±8.110
HIIT	One week after surgery	59.568±5.095	27.421±3.120
	Ten week after surgery	77.461±7.022	41.625±6.847
Control	-	91.493±2.440	57.878±3.943

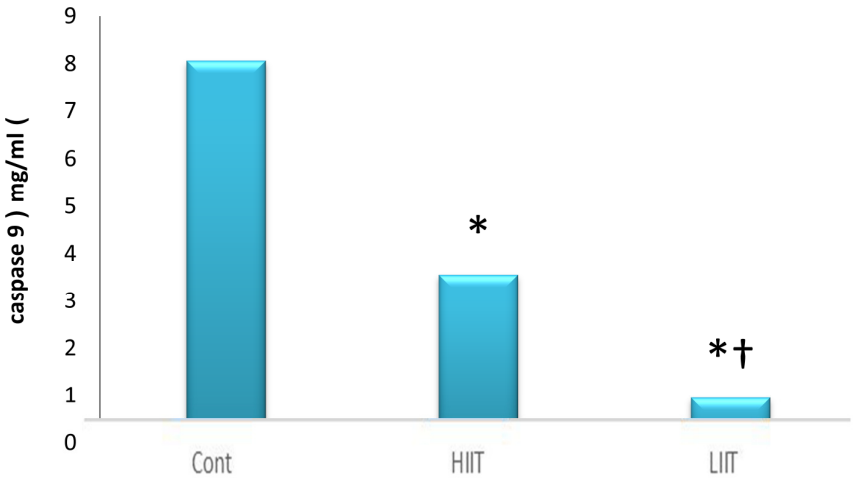

Figure 1. The caspase-9 means value in different groups (mg/ml)
*** Shows significant difference compared to the control group.**
† Shows significant difference compared to the high intensity interval group

training. Time and duration of training can be the source of difference. In the present study, it seems that high intensity interval training and low intensity interval training both induce factors effective in reducing the expression of caspase-9.

Past studies demonstrated that both training intensities are effective in increasing the mitochondrial biogenesis as well as improving their performance (14,15,16,17,18) and LIIT is more effective. One important biological role of the mitochondria is apoptosis. Mitochondria is the location of many proteins in the early stages of apoptosis process (19). It seems that six weeks of HIIT as well as LIIT improves the mitochondrial function. Also this factor reduces caspase-9 as the most important motivating factor of apoptosis. Probably the apoptosis pathways and pre-apoptosis signal molecules are transferred to the mitochondria as a result of training and induced a series of temporary permeable pores in the mitochondrial outer membrane. This inhibits the release of cytochrome c as the main player of the activation process of mitochondrial caspase-9 waterfall which is in interaction with cardiolipin, the exclusive mitochondria phospholipid and reduces the activity of caspase-9 (20,21). Generally, the findings of this study support the positive effect of six weeks of HIIT and LIIT on the inhibition of caspase-9 and ultimately the apoptosis of cardiac cells after MI.

Conclusions

The results of this study showed that interval training reduces caspase-9 gene expression and thereby reducing cardiomyocyte apoptosis after MI, which depends on the training intensity. It is recommended that attention is paid to training intensity in cardiac rehabilitation. LIIT is more effective than HIIT. However, further studies are needed to examine other factors involved in the process of apoptosis.

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