A comparison of the effect of eight weeks of high intensity interval training on PGC-1α gene expression levels in the slow twitch (ST) and fast twitch (FT) muscles of rats with myocardial infarction

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Abstract

Introduction: One of the side effects of myocardial infarction(MI) is a change in slow contraction muscle phenotype to fast contraction due to decreased mitochondrial density. Mitochondrial biogenesis with its ability to create new mitochondria and increase mitochondrial density can minimize these complications. Therefore, the aim of this study was to investigate the effect of eight weeks of high intensity interval training on peroxisome-proliferator activated receptor γ coactivator (PGC-1α) gene expression levels in fast twitch (FT) and slow twitch muscle (ST) muscles in rats with myocardial infarction.
Materials and methods: For this purpose, 12 Wistar male rats with MI were divided into two experimental groups (30 minutes on a treadmill on a regular basis and 4 minutes running with a severity of 85-90% VO_2 max and two minutes of active recovery with 50% -60% VO_2max three days a week for eight weeks) and control (without exercise). The expression of PGC-1α genes was studied as an effective factor in mitochondrial biogenesis. Statistical data were analyzed with independent T-test in SPSS18.
Results: The results showed that the expression of PGC-1α genes increased significantly in ST (P=0.012) and FT (P=0.001) muscles in rats with MI. Also, this increasing in the ST muscles was significantly higher than those in the FT muscles (P=0.000).
Conclusion: Generally, eight weeks of high intensity interval training increase mitochondrial biogenesis in ST and FT muscles of MI rats through effect on the PGC-1α gene expression.

Keywords: Myocardial infarction, Mitochondrial biogenesis, Interval training

Introduction

Skeletal muscle atrophy due to mitochondrial dysfunction is one of the most important complications of myocardial infarction (MI) in the skeletal muscle (1). In general, blockage of arteries and impaired blood supply and oxygen delivery, as a result of MI, affect various tissues, including skeletal muscle. This condition reduces mitochondrial size and density, causing a change in the slow twitch muscle (ST) contraction phenotype to the fast twitch (FT) contraction as well as atrophy of the muscle fibers (2). Therefore, finding a way to minimize the consequences of this complication and even its relative treatment by increasing mitochondrial density and improving their function under the influence of a process

called mitochondrial biogenesis has always been considered. Mitochondrial biogenesis with its ability to create new mitochondria and increase mitochondrial density can minimize these complications (3). Mitochondrial biogenesis is affected by two categories of transcription factors, i.e., transcription factors involved in the process of mitochondrial biogenesis that regulate mitochondrial DNA transcription and proliferation, and transcription factors that regulate mitochondrial genes encoded in the DNA of the nucleus (4).

Peroxisome-proliferator activated receptor γ coactivator (PGC-1α) is the most important transcriptional activator and the key factor in mitochondrial biogenesis and is present in mitochondrial rich tissues. In the cold, as well as short and fast training, the expression of it increases. It is involved in the physiological control of mitochondrial function. It is not a transcription factor by itself, but it binds to a transcription factor and regulates the expression of mitochondrial genes located in the nucleus. Its expression is increased by AMPK (AMPK-activated protein kinase) and calcium-calmodulin-dependent protein kinase (CamK) (5). This protein is phosphorylated by P38MAPK (6) and by enhancing the NRF-1 signaling pathway, it enhances the expression of mRNA transcription factor A (mtTFA) and ultimately enhances mitochondrial biogenesis (7). Normally, there is a balance between mitochondrial biogenic stimuli and inhibitors, but this is always disrupted in physiological and pathological conditions such as ischemic heart disease. The role of regular physical activity in health has been well documented. Recently, taking the shortest possible time, high intensity interval training (HIIT) has been recommended to overcome the lack of time to participate in exercise, thereby increasing body activity and health.

This type of training is a powerful stimulus for cardiovascular and muscular adaptations which increases maximal oxygen consumption (VO_2 max), metabolism and exercise performance, decreases carbohydrate use and elevates fat dependency, improves insulin function, lowers blood pressure and improves cardiovascular fitness in cardiovascular and hypertensive patients, and recently has been considered as a training method to establish cardiovascular angiogenesis. Due to the effect of this training method on increasing skeletal muscle mass and muscle hypertrophy (8), it is likely that it may affect the process of mitochondrial biogenesis and increase the size and density of mitochondria.

Concerning the impact of endurance activities on the process of mitochondrial biogenesis, extensive studies have been conducted, most of which point to the positive impact of endurance activities on this process (1, 9, 10).

Little is known about the role of HIIT in inducing mitochondrial biogenesis, and regarding the impact of HIIT, there is no direct research abroad and at home due to the nature of this training on mitochondrial biogenesis in slow and fast twitch muscles and specifically in myocardial infarction patients. In a foreign study of the impact of HIIT, Hushino et al. reported a greater increase in PGC-1α in the red muscles (22%) compared to the white muscles (66%) after four weeks of HIIT (11). Also, Little in his study reported the positive role of one HIIT session in enhancing PGC-1α gene expression in the skeletal muscle (12). In Iran, Azizi in his study noted that eight weeks of HIIT significantly increased PGC-1α, but no comparisons were made between slow-twitch and fast-twitch muscles, nor were the participants involved with MI (13). Sharafi Dahr Ham also reported a positive effect of three weeks of HIIT on the expression of PGC-1α and mtTFA genes (14). It has been reported that during HIIT hypoxia is induced (15). Also, Tervinges in his research reported hypoxia induction during HIIT (16). Hypoxia is one of the contributing factors to increased expression of PGC-1 alpha levels (17). On the other hand, the role of HIIT in muscle

hypertrophy, which is one of the main drivers of the increase in PGC-1 alpha expression, has also been demonstrated (8). Considering the information provided regarding the factors affecting the mitochondrial biogenesis process during exercise and the results of previous studies suggesting a positive and significant relationship between HIIT and these factors, it can be hoped that this training method may be effective on mitochondrial biogenesis. Therefore, the purpose of this study is to investigate whether eight weeks of HIIT, as an independent variable in rats with MI, affect the mitochondrial biogenesis capacity of the ST and FT muscles through increased PGC-1 alpha gene expression as dependent variables.

Materials and methods

In this applied research study, 12 male Wistar rats (10 weeks old) were purchased as the statistical sample from Razi Vaccine and Serum Research Institute. Rats were kept in separate cages with free access to water and food packages according to the principles of laboratory animal care (NIH-publication) and 12-hour sleep and wake cycle. Subsequently, rats were operated on and their left anterior descending (LAD) artery was blocked, thus causing severe myocardial infarction. The anesthesized rats were subjected to Doppler echocardiography (US, GE Healtcare Brand) to ensure they had MI.

During this process, the left ventricular shortening fraction (FS) was measured partially. Rats with FS≤35% were selected as rats with MI. The rats then underwent cardiac recovery for two weeks after open surgery. In the third and fourth weeks, rats were introduced to a treadmill (Iran, Danesh Salare Iranian Brand) by walking slowly at a speed of 5 m / min for five minutes a day and four days a week. At this stage, all rats were able to perform activity and had no casualties. The rats' VO_2 max was measured by the maximal exercise testing, in accordance with the formula and table presented by Morten et al. (18), Wislov et al. (19) to estimate the initial running speed of rats (18 and 19). Running speed of each rat on the treadmill was calculated individually according to its maximum oxygen consumption (VO_2 max). Finally, rats were randomly divided into two groups of HIIT and control (CTRL), and then eight weeks of training protocol was administered. In both training groups, rats warmed up for five minutes at a speed of 5 m / min before the start of the main training phase. Rats' running speed gradually increased by 0.02 m / s per week and the treadmill slope was zero degree throughout the training period (18). In contrast, control rats (with MI) received no training.

HIIT protocol

The training protocol consisted of 30 minutes of interval running on the treadmill. Each alternation included 4 minutes of running at 90-90% of VO_2 max and 2 minutes of active recovery at 50-60% of VO_2 max. Training was performed three days a week for eight weeks (19) and rats warmed up at 40-50% of VO_2 max for 5 minutes before starting the main phase of training. Running speed was gradually increased to 02.0 m/s every two weeks.

At the end of eight weeks, rats were subjected to surgery, and samples of ST muscle (soleus) and FT muscle (finger extensor) tissues were taken to measure RNA of PGC-1α using qRT-PCR method. The samples were transferred to the Genetics Laboratory after freezing, where the above factors were measured using the Real Time PCR method. The data collected were analyzed by SPSS 18 software. The Kolmogorov-Smirnov test was used to determine the normality of the data. Independent sample T-test at the significance level of 0.005 was used to analyze the data.

Results

As shown in Table 1, the mean of PGC-1α index in the ST muscle was 1.32 times

higher in the experimental group than in the control group. The mean of AMPK index in the experimental group was 6.46 times higher than the control group (Table 1). The results of the Kolmogorov-Smirnov test showed that the distribution of data in both groups is normal in two variables (Table 2).

Table 1. Descriptive statistics of research sample on PGC-1α index in the slow twitch and fast twitch muscles

Index	Groups	Number	Minimum	Maximum	Mean	SD
PGC-1α (ST)	Control	6	1.62	5.61	3.615	1.610
	HIIT	6	2.64	6.91	4.775	2.061
PGC-1α (FT)	Control	6	1	1.5	1.2243	0.23561
	HIIT	6	3.22	4.17	3.9022	0.34839

PGC-1α: peroxisome-proliferator activated receptor γ coactivator. HIIT: High intensity interval training. FT: Fast-Twitch Muscle. ST: Slow-Twitch Muscle.

The results of independent sample t-test showed that there was a significant difference between the control and experimental groups in PGC-1α in the ST muscle (P = 0.012), and according to Table 2, PGC-1α levels in the ST muscle were higher in the experimental group than in the control group (Table 2). There was also a significant difference between the control and HIIT groups in the PGC-1α level in the FT muscle index (P = 0.001). According to Table 2, the levels of the PGC-1α index in the FT muscle were higher in the HIIT experimental group than in the control group (Table 2). Also, the results of independent sample t-test showed that there was a significant difference between PGC-1α levels in the ST and FT muscles (P = 0.000) and according to Table 3, PGC-1α levels of ST muscle were higher than those of FT muscle (Table 3).

Table 2. Results of independent sample t-test to compare the control group and HIIT group in PGC-1α index in the slow twitch and fast twitch muscles.

Index	Group	Kolmogrov-Smirnov test		Independent T-test		
		P value	Result	Statistic (t)	Degree of freedom	Significance
PGC-1α (ST)	HIIT/Control	0.2/0.2	Normal/Normal	-3.835	5.089	0.012
PGC-1α (FT)	HIIT/Control	0.053/0.157	Normal/Normal	-0.049	10	0.001

PGC-1α: Peroxisome-proliferator activated receptor γ coactivator. HIIT: High intensity interval training. FT: Fast-twitch muscle. ST: Slow-twitch muscle.

Table 3. Results of independent sample t-test to compare PGC-1α index in the slow twitch and fast twitch muscles.

Type of Muscle	Test Statistic	Degree of Freedom (df)	Level of Significance
ST/ FT	7.813	10	0.000

PGC-1α: peroxisome-proliferator activated receptor γ coactivator. FT: Fast-Twitch Muscle. ST: Slow-Twitch Muscle.

Discussion

The results of this study showed that eight weeks of HIIT increased mitochondrial biogenesis in both ST and FT muscle fibers and this increase was more in the ST fibers than in the FT fibers. Although no research has been found to directly examine the impact of HIIT on mitochondrial biogenesis in the ST muscle in patients with MI at home or abroad, the results of this study are consistent with the findings of Little et al. (12), Hushino et al. (11), Azizi et al. (13) and Sharafi Dahr Ham et al. (14).

As noted, PGC-1α is a focus of mitochondrial biogenesis that is enhanced by upstream factors such as NRF-1, NRF-2 and AMPK and induces mitochondrial biogenesis by affecting downstream factors such as MtTFA. It appears that in the present study, six weeks of HIIT induces

factors that affect increased PGC-1α gene expression including hypoxia and increased ROS, thereby stimulating mitochondrial biogenesis in both ST and FT fibers. The stress induced by HIIT as a strong stimulus is likely to cause vasodilatation and increased blood flow to the muscles and, by improving calcium release (Ca^{2+}) due to decreased mitochondrial ATP density, in addition to increased cytosolic calcium, enhances mitochondrial matrix calcium density, which increases calcium levels sufficiently and activates matrix dehydrogenases. A downstream effector kinase on the calcium signaling pathway, i.e., calcium-calmodulin-dependent protein kinase, enhances mitochondrial DNA replication and mitochondrial production along with over-regulation of mitochondrial enzymes. This effect is mediated by PGC-1α gene expression. In general, increasing CaMK levels and calcium levels of the endocarcoplasmic reticulum network by affecting upstream factors of mitochondrial biogenesis increases AMPK-activated protein kinases such as PGC-1α (5).

Levels of eNOS probably increased as a result of eight weeks of HIIT which increase cGMP and p38 levels due to increased ROS (20). Changing intracellular levels of ATP / ADP / AMP following exercise increase p38 (21), which activate MAPK and thereby increase PGC-1α expression (11, 22). Increased expression of PGC-1α as a result of increased AMPK will eventually increase mtTFA expression and regulate mitochondrial DNA and mitochondrial genes encoded in the nucleus (23).

Conclusion

In general, all transcription factors, in particular PGC-1α, bind to and regulate the expression of mitochondrial genes encoded in the nucleus, ultimately inducing mitochondrial biogenesis in both slow twitch and fast twitch muscle fibers (23) which is accompanied with increased mitochondria and increased aerobic function in both ST and FT muscle fibers of rats with MI.

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