

The Effect of Moderate and High Intensity Interval Trainings on Cardiac Apoptosis in the Old Female Rats

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Received: 8 November 2017

Accepted: 3 February 2018

Published online: 1 March 2018

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Competing interests: The authors declare that no competing interests exist.

Citation: Yazdanparast Chaharmahali B, Azarbayjany MA, Peeri M, Farzanegi Arkhazloo P. The effect of moderate and high intensity interval trainings on cardiac apoptosis in the old female rats. Report of Health Care. 2018; 4 (1): 26- 35.

Abstract

Introduction: Aging causes apoptosis in the heart. While the old heart is vulnerable to apoptosis, physical activity with different mechanisms can be effective in reducing apoptosis or inhibit it. The purpose of this study was to investigate the effect of moderate and high intensity interval trainings on some apoptotic indices of Bax and Bcl- 2 cardiomyocytes of old female rats.

Methods: In this study, 21 aged female, postmenopausal Wistar rats (2 years old), were randomly divided into three groups: 1) control, 2) moderate intensity interval training (MIIT), and 3) high intensity interval training (HIIT). The training protocol performed consisted of two different intensities (MIIT and HIIT) of running on treadmill 3 sessions per week for 8 weeks. 48 hours after the last training session, the heart tissue was removed in order to analyze Bax, Bcl- 2 genes and Bax/Bcl- 2 ratio in the cardiomyocytes. Two-way analysis of variance was used to determine the main effect of training. In case of a statistically significant difference, Bonferroni post hoc test was used to determine the intergroup difference. Significance level for all data analysis was considered as $p \leq 0.05$.

Results: Bax ($p=0.0001$) and Bax/Bcl- 2 ratio ($p=0.001$) gene expression was higher in the HIIT group in comparison with MIIT group nevertheless Bcl- 2 gene expression was higher in the MIIT group in comparison with the HIIT group ($p=0.0001$).

Conclusion: Based on the present results, treadmill exercise with MIIT can support cardiac against apoptosis through the enhancement of Bcl- 2 expression in the heart. Therefore MIIT may be a beneficial method for preventing heart problems in the aging.

Keywords: Cardiac Apoptosis, Training, Rat, Aging

Introduction

Aging has been described as diminished general physiological function (1). Reducing heart cells is an important mechanism in heart deformity and heart failure (2). These are the gradual changes in the performance of a living creature that eventually cause death. It is believed that aging is developed due to reducing heart function including stroke volume, cardiac output, bloodstream, oxygen absorption, and increased susceptibility to inflammation, oxidative stress, and disease. With cardiac malfunctioning, inflammatory cytokines grow, and elevated oxidative stress is common in aging and cardiovascular

diseases (3). It is believed that a significant reduction in the number of cardiac cells indirectly contributes to diminished contraction function, myocardial diseases, other diseases, and heart failure (4). The loss of cardiac cells with aging occurs due to necrosis or apoptosis, both of which cause cellular death (5). Apoptosis is a programmed cellular death which is without damage or inflammation, and is characterized by shrinkage of cell nucleus (6). Zhang (2003) believes that in humans, apoptosis is responsible for maintaining the balance between cell proliferation and cell death, and keeping the number of cells constant in the

tissue (7). Apoptosis plays a significant role in controlling the progress, growth, and replacement of cells (8). Although excessive apoptosis has been reported in diseases and lack of heart function, increased apoptosis due to aging has been demonstrated in the left ventricle of rats (5). The progress of apoptosis with aging is horrible in tissues such as heart; this is because the dead cells are not replaced. Unfortunately, the mechanisms responsible for apoptosis and its signaling pathway in old hearts still remain unknown and poorly understood (6). Studies have shown that aging is associated with elevated Bcl-2-associated X protein (Bax) and increased DNA damage in the brain (6). It has been reported that expression of Bax and B-cell lymphoma 2 (Bcl-2) proteins is increased in the heart of old rats. Nitahara (1998) reported increased apoptosis in cardiac cells due to aging in rats without aging related significant changes in Bax and Bcl-2 levels (9). These findings suggest that age-associated changes in the expression of family gene Bax and Bcl-2 are still unknown (9). In response to an apoptotic stimulus, a wide range of internal and external signals regulate expression of genes, thus controlling initiation of apoptosis. In the internal pathway, the genes cause expression of proteins, causing apoptosis initiation (e.g. Bax). On the other hand, the proteins that inhibit development of apoptosis (e.g. Bcl-2) and its development for cell (death versus survival) are dependent on the ratio of expression of these genes (9). Bcl-2 is an important protein inhibiting apoptosis as well as releasing of cytochrome C from mitochondria. Large amounts of Bcl-2 in relation to Bax cause cell survival, whereas its reverse ratio leads to cellular death (10). Cellular death causes cardiac breakdown. Many studies have shown that apoptosis of cardiac cells plays a significant role in myocardium breakdown and lack of cardiac function. Apoptosis is a kind of cellular death which causes special morphological changes and DNA damage, whose important sign is

apoptosis (10). Physical training is the key prescription regarding physiological adaptation without alternative for heart and cardiac diseases (11). In recent years, endurance sports have been recognized as the shield against ischemia-reperfusion (IR), which is considered a special aspect of endurance sport mechanism on cardiac protection (12). Indeed, recent findings indicate that regular training protects the heart against apoptosis. Physical activity, by enhancing catalase activity of cardiac cells, may help in preventing apoptosis of cardiac cells resulting from IR, considering that Glutathione peroxidase (GPX), Mn superoxide dismutase (SOD), and copper (Cu)/zinc (Zn) activities increases due to physical activity (12). Physical training has the potential of reducing apoptosis through protecting stress sensitive proteins including nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B), Insulin-like growth factor 1 (IGF-1), and heat shock protein (HSP70). Kwak has indicated that Bax/Bcl-2 ratio is regulated with age, and it specifically declines with physical training in the left ventricle (13). This information suggests that the elderly's heart is more susceptible to apoptosis compared to younger hearts. Further, physical training brings about anti-apoptotic activity in the elderly's heart (13). Recent studies have indicated that physical training reduces expression of Bax and caspase, but it enhances Bcl-2 expression, which is an anti-apoptotic protein in both muscular and cardiac cells of healthy rats. The results of a clinical study revealed that physical training diminishes apoptosis of muscular cells in the long-term, causing enhanced antioxidant capacity in cardiac patients (14). Various studies have reported that physical training acts as an effective intervention in the prevention of primary and secondary cardiovascular diseases, though the nature, duration, and intensity of exercises that are useful to the cardiovascular system are still unknown (15). Physical activity has the potential of

modifying proliferation and death of cells through cytokines, hormones, growth factor, and metabolic pathways. Recently, some evidence has been found for apoptosis resulting from physical training, which occurs in lymphocytes and skeletal muscle (11). Physical training may have protective effects in reducing oxidative stress, disorder in mitochondria, and apoptosis associated with mitochondrial caspase with aging. Studies have revealed that physical activity causes healthy heart function and prevents development of atherosclerosis (16,17). However, the effect of intensity of training on cardiovascular health has rarely been investigated, and as such further studies should be conducted. Thus the aim of this study was to examine the effect of different intensities of interval training on some of the genes associated with cardiac apoptosis in old rats.

Methods

21 female Wistar rats, aging 2 years old, with the initial mean weight of 250- 300 gr, were purchased by the research and reproduction of laboratory animals center, Islamic Azad University, Sari Branch. The rats were housed in transparent polycarbonate cages (30 × 15 × 15 cm) made by Razi Rad Company, lighting to darkness cycle of 08:00 to 20:00 O'clock, under controlled temperature of 22± 2°C and air humidity of 50±5%, equipped with a suitable ventilation. The mean and standard deviation of the weight of rats are presented in Table 1. Rats food were placed inside the cages by weighing once every three days using a special standard scale and based on natural ration of 10 gr per 100 gr of the bodyweight. During all stages of the research, the animals had free access to water. From the total population (42 rats), 21 rats were chosen and after entering the research environment and following one week of adaptation with the new environment, animals were divided into three equal groups including control, MIIT and HIIT (n= 7 in each group). After being transferred to the laboratory, rats underwent a 5- day

treadmill training adaptation program consisting of 5- min treadmill training a day. Thereafter, one training session was held to measure the maximum extent time to exhaustion, with a mean of 28 m per minute. The MIIT involved eight weeks, three sessions per week, and each session 10 sets of 1-min activity with 50 % of the exhaustion point intensity and 2 min inactive rest between the sets. The first week began with 14 m/min, and each week 2 m/min was added to the speed, finally reaching 28 m/min in the eighth week. HIIT program was also performed similar to the moderate exercise, but with 70 % of the exhaustion intensity. In the first week, it began with 20 m/min, and each week 2 m/min was added to the speed, finally reaching 34 m/min in the eighth week. Furthermore, five minutes warm up and the same time for cooling down sessions before and after the training, respectively were considered for the animals. In this way, the duration of each training session was fixed to 40 min throughout the whole research. The training protocol was implemented by a fully automatic treadmill. Table 2 reveals the intensity of training in each week across the experimental groups. Eight weeks after the research implementation, with absolutely the same conditions and after 12-14 hours of fasting and 48 hours after running the last training session, all animals were anesthetized by intraperitoneal injection of ketamine from Netherland Aphasun compony (6 mg per each kilogram of the weight of rats) and xylazine (5 mg per each gram of the weight of rats), and after ensuring complete anesthesia by testing corneal reflects, the chest was excised and the cardiac tissue was separated. RNA extraction from the cardiac tissue was performed using Qiazol (Qiagen kit, Germany) according to the manufacturer's recommendation. To eliminate the possibility of RNA contamination with DNA, DNAase enzyme free from RNAase was used. The required amounts were determined based on the concentration of extracted RNA. Accordingly, per every 1 mcg of extracted

RNA, 1 mL (DNase Ferment ase) and 1 mL buffer 10X were added, and the solution volume was brought to the volume of 10 mL using water treated with DEPC. The resulting solution was exposed to a temperature of 65°C for 15 min in order to deactivate the enzyme. RNA concentration was determined using spectrophotometric method (UV Eppendroff, Germany). To fabricate cDNA, 1 mL of oligo dt was added to 0.2-1 mcg of extracted RNA. The final volume of this stage should be 12 mL. Accordingly, if RNA was more concentrated, less of it would be withdrawn, with water treated with DEPC, it was brought to a final volume of 12 mL. The reaction was subject to a temperature of -70°C for 5 min, and then immediately transferred to ice. Next, 4 mL of buffer 5X, 2 mL of dNTP, and 1 mL of Rnase were added to the microfiuge to obtain a final volume of 19 mL. The reaction solution was incubated at a temperature of 37°C for 5 min. Thereafter, 1 mL of RT enzyme was added to the reaction and then incubated at a temperature of 42°C for 1 h. To stop the reaction, the micro tube was exposed to a temperature of 70°C for 10 min. The resulting CDNA was placed on ice, and prior the polymerase chain reaction (PCR) reaction, it was kept in the fridge at a temperature of -20°C. To design the primers, first the mRNA sequence related to Bax and Bcl- 2 and Caspase 3 genes was extracted using NCBI site. The primers were developed by Allel ID software, and then each primer was evaluated by BLAST software to ensure the unity of the site of primers coupling. The primers were synthesized by Sinagen Company. In this research, GAPDH gene was utilized as internal control. Each PCR reaction was performed using PCR master mix Applled Biosystems, SYBR Green in ABI STEP ONE DETECTION APPLIED BIOSYSTEMS, SEQUENCE CA SYSTEMS, FOSTER CITY device according to the manufacturer's protocol. A total of 40 cycles were considered for each real-time PCR cycle. The temperatures of every cycle Scarf cycle were

adjusted to 94°C for 15 s and 60°C for 30 s. For all the studied genes, the reference gene (GAPDH) was performed to obtain the suitable annealing temperature gradient. Furthermore, to investigate the efficiency of primers, the specific standard curves of each gene (DNA diluted series) were plotted. MELTING diagram was also drawn to investigate the actors of the PCR reactions specifically for each gene, which was evaluated in each time of reaction. Their reference genes were almost equal. By embedding the data in $\Delta\Delta C1$ and $-2\Delta\Delta C1$ formulas, the extent of expression of the target gene was normalized with reference genes. In each stage of the procedure, gene expression of blastocysts of the control group was considered as the calibrator. All of the statistical operations were performed by SPSS 22. In the descriptive part, mean and standard deviation were used. In the inferential) statistics part and hypothesis testing, the obtained results were analyzed. In case of a statistically significant difference, Bonferroni post hoc test was used to determine the intergroup difference. Significance level for all data analysis was considered as $p \leq 0.05$.

Bax R	CAG CCA CAA AGA TGG TCA
Bax F	GCA AAC TGG TGC TCA AGG
Bcl2 R	TTTTGCTGCTCACTGTATTTTATTTT
Bcl2 F	ACTTTTAGGCGTGGCTGATG

Results

Training had a significant effect on the expression of cardiac Bax gene ($p=0.003$), such that its expression was higher in the HIIT group in comparison with MIIT group ($p=0.0001$); as shown Figure 1. Based on Fig 2, training had a significant effect on the expression of Bcl- 2 gene ($p=0.001$), such that its expression was higher in the MIIT group in comparison with the HIIT group ($p=0.0001$); as shown in Figure 2. Training had a significant effect on Bax/Bcl- 2 ratio ($p=0.001$), such that its expression was higher in the HIIT group in comparison with the MIIT group ($p=0.001$); as shown in Figure 3.

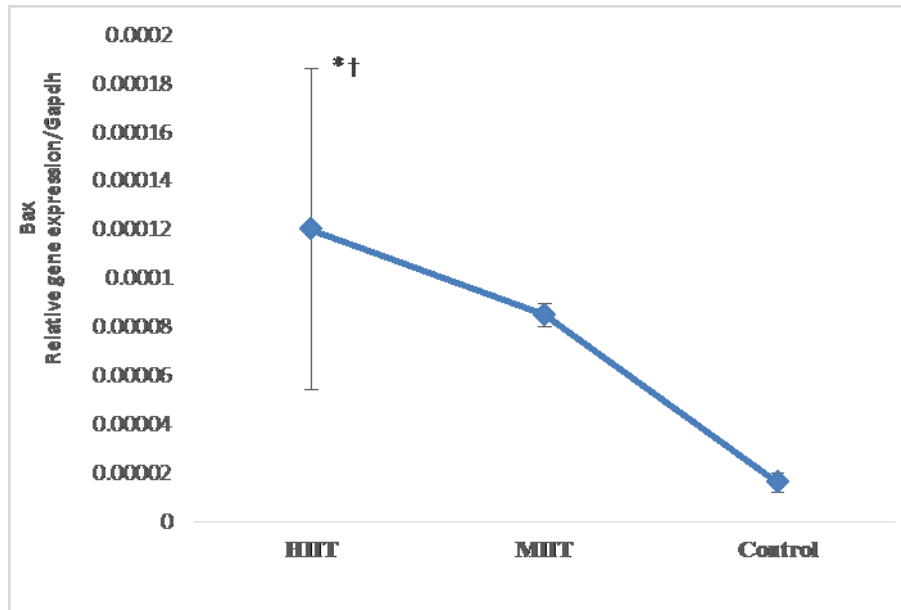


Figure 1. Expression of cardiac Bax gene across the studied groups
* Significant increase rather than control group
† Significant increase rather than MIIT group

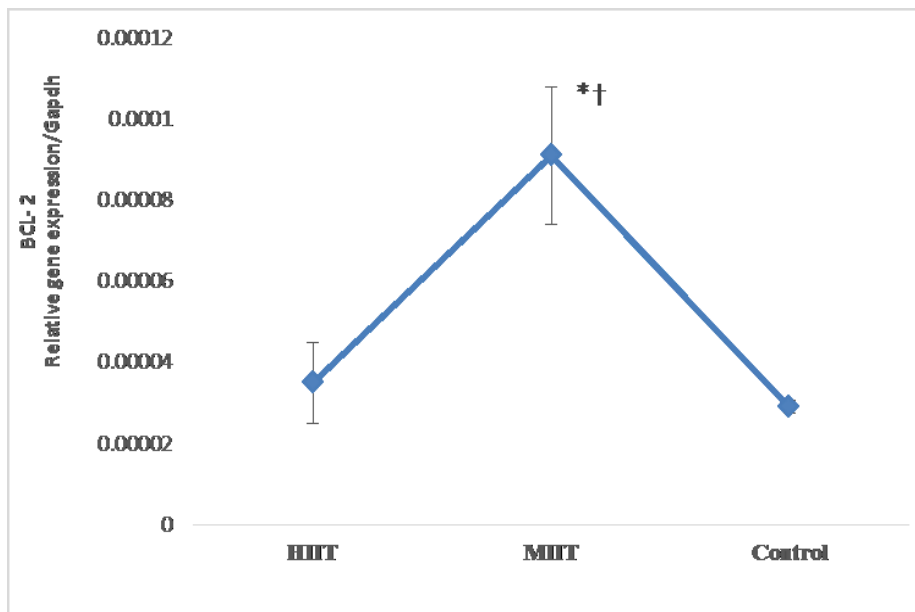


Figure 2. Expression of cardiac Bcl- 2 gene across the studied groups
* Significant increase rather than control group
† Significant increase rather than HIIT group

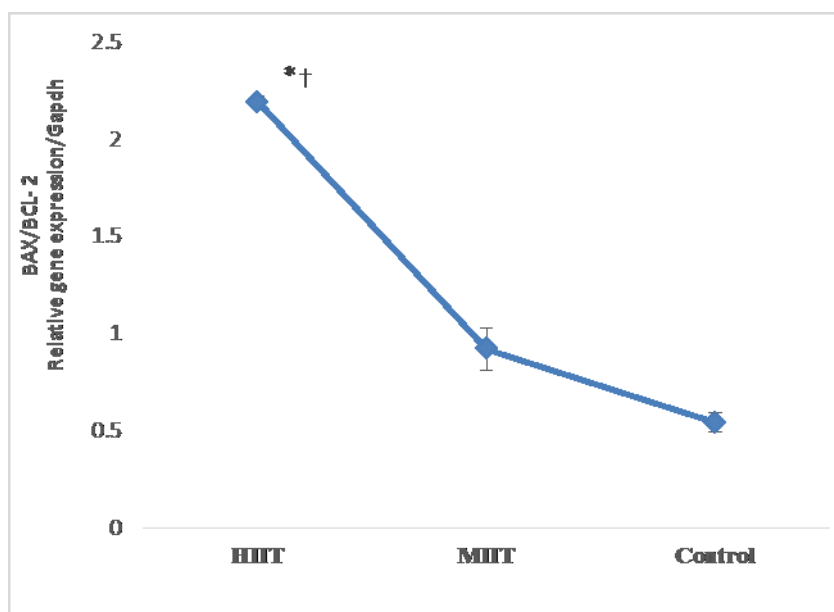


Figure 3. Cardiac Bax/Bcl- 2 ratio across the studied groups

* Significant increase rather than control group

† Significant increase rather than MIIT group

Table 1. The mean and standard deviation of the weight of rats across the research groups

Group	Weight (gr)
Control	255.5 ± 1.4
MIIT	265.38 ± 27
HIIT	264.39 ± 33.66

MIIT: Moderate Intensity Interval Training

HIIT: High Intensity Interval Training

Table 2. The intensity of training in each session

HIIT (m/min)	MIIT (m/min)	Week
20	14	Week One
22	16	Week Two
24	18	Week Three
26	20	Week Four
28	22	Week Five
30	24	Week Six
32	26	Week Seven
34	28	Week Eight

MIIT: Moderate Intensity Interval Training

HIIT: High Intensity Interval Training

Discussion

The results of this study indicated that the indicator supporting apoptosis Bax and the anti-apoptotic indicator Bcl- 2 had higher expressions in response to the effect of interval training. In this regard, HIIT caused increased expression of Bax and Bax/Bcl- 2 ratio, while MIIT resulted in diminished expression of apoptosis supporting indicators and Bcl- 2 indicator. With aging, the extent of cardiac cell death also increases. Aging is a complex process associated with changes in the physiological, morphological, cardiovascular all of which are involved in the development of two important patterns of cellular death. A number of studies have reported that in old cardiac tissue cells, expression of P16/INK4A and P53 genes increases, of which simultaneous with alteration in the telomerase enzyme, aging begins in the cell and apoptosis develops (18,19). Furthermore, a number of studies have reported that during senescence, apoptosis occurs due to diminished function of Sarcoplasmic Reticulum *ca* ATPase enzyme and reduced expression of Bcl- 2 and surviving (20). Caspase 3 functions as the key. Bcl- 2 is necessary for apoptosis process resulting to elimination of cellular death process. Furthermore, Bax gene has been identified as the first member of Bcl- 2 family supporting apoptosis (21). Li *et al.* (2013) reported that physical training can prevent apoptosis as a new drug (22). Physical training prevents the activity of mitochondrial-dependent apoptotic pathway, which is characterized by increased expression of Bcl- 2 and Bax and cardiac Bax/Bcl-2 ratio, declined caspase 9 activity, and lack of caspase 3 activation (22). Previous studies have reported that physical training reduces Bax expression, caspase 3 activity, and DNA damage in myocardium, though no change was developed in the skeletal muscle of fat rats (23). These findings indicated that physical training supports the heart against apoptosis (22). Physical training prevents apoptosis through mitochondrial pathway. The proteins

of Bcl- 2 family are the intermediates of the mitochondrial pathway of apoptosis, which prevent release of cytochrome C from the mitochondria (20). Elevation of Bax/Bak, ROS, and excessive calcium ions cause release of cytochrome C from mitochondria to cytosol. Therefore, it seems that physical activity could be effective in preventing cardiac apoptosis by lowering ROS to some extent and preventing release of mitochondrial cytochrome C (20). The effect of physical exercise on the health of the heart of patients may be due to various factors including diminished lipotoxicity, strengthened myocardium, improved antioxidant capacity and antihypertensive capacity (24, 25). Therefore, the therapeutic effects of physical activity on cardiomyopathic changes and cardiac apoptosis can be attributed to different factors such as weight loss, changes in blood pressure, antioxidants, and other unknown factors (22). A progressive decline occurs in myocardial cells during senescence and heart disability, with both necrosis and apoptosis playing a significant role in this process. In addition, Kajustra *et al.* indicated that apoptosis in the left ventricle of 24-month rats was 100 % higher than that of 16-month rats, though no slight extent of necrotic cellular death was experienced. They contended that apoptosis may be more common than necrosis in old rats.

In the present study, training on treadmill with moderate intensity prevented Bax expression and increased Bcl- 2 expression in the old rats (26). Zhang *et al.* reported that slight variations in mitochondrial DNA observed in senescence enhance apoptosis in old rats. Fahnenhof and Lewenberg reported that Bax and Bcl- 2 play an important role in mitochondrial apoptosis associated with aging (26). Bcl- 2 membrane inhibits apoptosis by preventing release of cytochrome C from mitochondria. Cellular death is also prevented due to increased Bcl- 2/Bax ratio. Elevation of this ratio prevents release of cytochrome C and activation of caspase 3, leading to diminished

apoptosis (27). In their study, mitochondrial DNA mutation in the rat heart resulted in saver response, including overregulation of Bcl- 2. Both programmed activities for saving the cell and its death may occur in the heart of old rats. The beneficial effect of physical activity on myocardium for old rats involves preventing expression of Bax and elevation of Bcl2, which occurred in the research. Therefore, regular exercise could be a useful strategy to prevent cardiac problems in the elderly (27). Li *et al.* (2000) reported that apoptosis-dependent cardiac receptors in fat rats were less active after 12 weeks of physical training. Further, the mitochondrial-dependent apoptotic pathway diminished significantly after 12 weeks. Also, Bcl- 2 apoptotic opposite protein increased, while Bax as well as Bax/Bcl- 2 decreased (22). Siu *et al.* revealed that endurance training causes downregulation of caspase and Bax, but upregulation of Bcl- 2 in both skeletal and cardiac muscles in mice. These anti-apoptotic effects have been illustrated with increased Mn-SOD protein value. A clinical study indicated that physical training causes apoptosis weakening in the skeletal muscle together with improved antioxidant capacity and lowered oxidative stress, and physical training may cause reduction of apoptosis supporting genes. Apparently, physical activity causes diminished apoptosis (14).

Conclusion

The present findings indicated that moderate intensity interval training for eight weeks significantly reduced apoptosis such that it caused increased Bcl- 2 (apoptosis supporting protein) and reduced expression of Bax and Bax/Bcl-2 ratio. It has also been shown that moderate intensity training led to reduced apoptosis in old rats. The result can be used in preventing and preserving heart health among the elderly and in rehabilitating cardiac patients.

Ethical issues

Not applicable.

Authors' contributions

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

Acknowledgements

This paper from a PhD dissertation on the physical education of sport physiology in Islamic Azad University, Central Tehran Branch with code No. 10121404961004.

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