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Protective Effects of High-Intensity Versus Low-Intensity Interval Training on Isoproterenol-Induced Cardiac Injury in Wistar Rats

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Abstract

Background: Cardiovascular diseases are among the major causes of mortality in industrialized countries. Prevention of cardiovascular diseases and increasing stress tolerance are two of the main goals of physical training.
Objectives: This study was designed to compare the effects of two exercise programs of different intensities on rat hearts with isoproterenol-induced myocardial injuries.
Methods: Animals were randomly divided into four groups (n = 8 per group): control group (CTL); ISO group, administered isoproterenol (85 mg/kg subcutaneously) for two consecutive days; low-intensity interval training + isoproterenol group (LIIT + ISO: 5 minutes warm up at 40% VO_2 max, 5 × 10 minutes at 50% - 60% VO_2 max [about 20 - 24 m/min]); and high-intensity interval training + isoproterenol group (HIIT + ISO: 5 minutes warm-up at 40% VO_2 max, 5 × 5 min at 95% - 105% VO_2 max [45 - 50 m/min]). The training groups performed high- and low-intensity interval training programs (5 days/week) on a motor-driven treadmill for 16 weeks. Seventy-two hours after the last training session, isoproterenol (85 mg/kg) was injected on two consecutive days. On the third day, hemodynamic parameters were recorded, blood samples were taken, and hearts were removed for laboratory analysis.
Results: ISO-induced heart injury raised cardiac troponin I levels, significantly decreased + dp/dt max ($P < 0.05$) and -dp/dt max ($P < 0.05$), and significantly increased serum CTnI and tissue TNF α levels ($P < 0.05$). Exercise training had no significant effects on HR, LVSP, and LVEDP. Impairments of + dp/dt max and -dp/dt max were significantly improved in the HIIT + ISO and LIIT+ISO groups ($P < 0.05$ for both groups versus ISO). In addition, exercise training groups especially HIIT + ISO to some reduce exacerbated the myocardial lesions induced by ISO ($P < 0.05$).
Conclusions: These biochemical and histopathological findings suggest there is a protective role provided by both high- and low-intensity interval training protocols on ischemic hearts.

Keywords: Training Intensity, Isoproterenol, Myocardial Injury

1. Background

Cardiovascular disease (CVD) is the major cause of disability and early death in both developed and developing countries (1). In this regard, coronary heart disease (CHD) makes up more than half of all cardiovascular events in the American population for those who are under 75 years of age (2). Consequently, several strategies, pharmacological or not, have been proposed to induce cardioprotection. Among these, regular exercise has been shown to reduce cardiovascular-induced morbidity and mortality rates (3, 4). The lack of continuous physical activity causes undesirable complications in the heart. Under these conditions, any challenge, stress, or unsuitable physical condition can bring undesirable outcomes for heart health. A literature review by Calvert and Lefer (2013) showed that following all exercise types, cardiac cell death is reduced by 4% - 75%, and they concluded that exercise training reduces infarct size by an average of 34% in animal models (5). Human and animal studies have shown the protective effects of regular exercise on heart functions (5-12). The anatomical, biochemical, and neural adaptations induced by exercise have been called "exercise preconditioning" (EP) (4, 11, 13). However, training protocols with different types and intensities of exercise have shown different endogenous and protective responses. Moreover, the existing evidence recommends that exercise intensity, rather than duration and frequency, is the most critical factor determining exercise-induced cardioprotection (EICP) (14-17). Undoubtedly, EICP vanishes after the termination of exercise training (18). However, how long would it take for the EICP against ischemia/reperfusion (I/R) to disappear once an exercise program is terminated? In this context, Lennon et al. (2004b) reported that after 3 days of continuous endurance training (CET) (60 minutes, 30 m/min, ~ 70% VO_2 max), protection against myocardial stunning remained up to 9 days and was lost 18 days after exercise termination. Calvert et al. (2011) (12) reported that after 4 weeks of voluntary exercise (~7.4 ± 0.2 km/d); EICP against I/R was only retained for a week after detraining. Some studies have reported

improvements in the heart's resistance to ischemia following both moderate-intensity (19) and low-intensity training (20); however, another study reports an ineffectiveness of low-intensity training on the heart's resistance to ischemia (21). Certain other studies have also shown undesirable physiological responses including increases in cardiac troponin I (cTnI) release from heart tissue in response to high-intensity training (22-24). Although the beneficial effects of exercise are clear, the effects of training intensity and the resulting EP effects against heart ischemia and the heart's physiological responses and functions remain unclear. A specific HIIT-Rx or LIIT-Rx recommendation to attenuate or prevent unfavorable health risks becomes difficult to establish due to several combinations that could result from the manipulation of HIIT and LIIT components. These factors include: (1) the number of interval bouts, (2) the intensity and duration of each bout, (3) the types of recovery periods, (4) the number of sessions per week, and (5) the duration of the program (25).

2. Objectives

Training protocols with different intensities may have different preconditioning effects. Therefore, this study was designed to investigate the effects of training intensity on the resistance of the heart to experimentally-induced ischemia through studying heart function, and physiological and histopathological indices.

3. Methods

3.1. Animals and Experimental Design

All experimental procedures were carried out in accordance with the national guidelines for conducting animal studies (ethics committee permission No 91/171KA-Kerman University of Medical Sciences, Kerman, Iran).

Animals were divided randomly into four groups: control (CTL), ISO, HIIT+ISO, and LIIT+ISO. In the CTL group, animals received equivalent volumes of distilled water intraperitoneally (ip) as ISO solutions for two consecutive days. The isoproterenol (ISO) group received 1 mL/kg distilled water ip 30 min before ISO (85 mg/kg subcutaneously (sc)) for two consecutive days. The low and high-intensity interval training groups (LIIT + ISO and HIIT+ISO, respectively) were trained with programmable treadmills adapted for rats (Medical Development-France).

3.2. Training Protocol

Exercises were carried out for 16 weeks (5 times/week). The habituation period to the treadmill (2 weeks) involved a gradual increase in running time, beginning with 10 minutes of running and ending with 25 min/day at 15 m/min on a 5% grade. The two exercise groups included the LIIT group (50% - 55% VO_2 max) and HIIT group (90% - 95% VO_2 max). The exercise intensity was estimated based on the rate of oxygen consumption rather than on the maximal oxygen consumption. The rate of oxygen consumption was 50% - 55% and 90% - 95% for the LIIT and HIIT groups, respectively (26, 27). The work rate of the interval training protocol was assimilating in total of exertion period in animal of both training groups. The principle of progressivity in load was applied with changes in speed and time of the exercises for the HIIT and LIIT groups, respectively. In the LIIT group, the speed started at 16 m/min and finished at 24 m/min, and in the HIIT group, the speed changed from 22 to 45 m/min (Table 1).

3.3. Surgical Preparation and Experimental Protocol

At the end of the training period, in order to induce myocardial infarction, isoproterenol hydrochloride (Sigma Aldrich, Germany) was dissolved in saline and injected (85 mg/kg, sc) over two consecutive days with a 24-hour interval between injections (28). On the third day, animals were anesthetized by sodium thiopental (50 mg/kg of body weight, ip) and after deep anesthesia was induced, the trachea was cannulated with the animals breathing spontaneously throughout the experiment. The left carotid artery and left ventricle were cannulated by two heparinized saline-filled (7 units/mL) catheters that were connected to pressure transducers and a physiograph (Beckman R612, USA). The time window for animal recovery from surgery was 30 minutes. Then, electrocardiogram (ECG), systolic and diastolic blood pressures, left ventricular systolic pressure (LVSP), and left ventricular end-diastolic pressure (LVEDP) were recorded. The mean arterial pressure (MAP) was calculated by: MAP = Pd + (Ps - Pd)/3, where Ps and Pd are systolic and diastolic arterial pressures, respectively. The maximum velocity of contraction (+ dp/dt max) and maximum velocity of relaxation (-dp/dt max) were calculated from the left ventricular pressure trace (29). Thereafter, blood samples were taken for measurement of serum cTnI levels, which is an important biomarker for the estimation of myocardial injury. Homogenization was done on an ice-cold buffer and a protein concentration of the supernatant was measured using the Lowry method. Rat tumor necrosis factor (TNF)-α platinum enzyme linked immunosorbent assays (ELISA) and Kamiya biomedical kits were used to measure TNF-α and serum cTnI levels of heart tissue, respectively.

Table 1. Training Protocols for the Exercise and Control Groups

Groups	Session Duration, (min)		Speed, (m/min)		Inclination, (Degrees)
	Start	End	End	Start	
LIIT	16	24	50	30	10
HIIT	22	45	26	22	10
Controls	15 - 20 meter walk for exposure to stress				10

Abbreviations: LIIT, low-intensity interval training; HIIT, high-intensity interval training.

3.4. Histopathological Study

The hearts were removed, washed with saline, fixed with 10% buffered formalin, and embedded in paraffin. Slides were prepared and stained with hematoxylin and eosin (H&E) and examined microscopically by two pathologists blinded to the animal groupings. The lesions were graded as 0: none, no damage or inflammatory processes; 1: minimum, focal myocyte damage; 2: mild, small multifocal degeneration with slight degree of inflammatory processes; 3: moderate, extensive myofibrillar degeneration and/or diffuse inflammatory processes; and 4: severe, necrosis with diffuse inflammatory processes (30).

3.5. Statistical Analysis

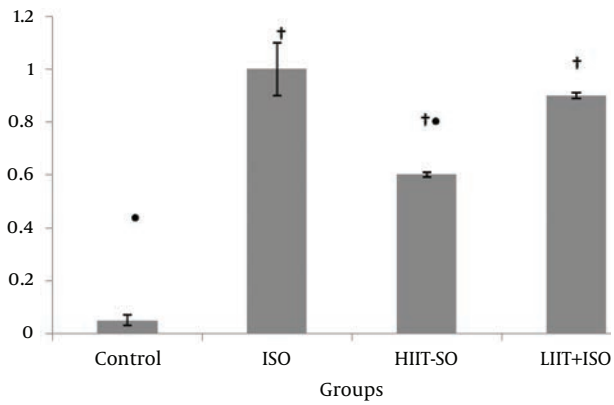
Quantitative data are expressed as mean ± standard error of the mean (SEM) and comparisons were performed by a one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) post hoc test. Histopathological changes are reported qualitatively as the number of animals with different grades of myocardial lesions in each group and statistical analysis was performed using the non-parametric Kruskal-Wallis test, while pairwise differences were determined with Mann-Whitney U tests. $P < 0.05$ were considered statistically significant.

4. Results

4.1. Hemodynamic Parameters and Ventricular Function Indices

A non-significant decrease was observed in heart rate (HR), Ps, Pd, and MAP of the CTL group versus other groups (Table 2). LVSP and LVEDP decreased in ISO, HIIT + ISO, and LIIT + ISO groups compared to the CTL group ($P < 0.001$). ISO caused tissue damage in all groups and significantly increased serum cTnI levels. Serum cTnI decreased in the LIIT + ISO, HIIT + ISO, and CTL groups compared to the ISO group, but this decrease was significant in only the CTL and HIIT + ISO groups ($P < 0.01$ and $P < 0.003$, respectively) (Figure 1). Tissue levels of TNF-α also decreased in the CTL, HIIT + ISO, and LIIT + ISO groups compared to the ISO group ($P < 0.001$, $P < 0.001$, and $P < 0.03$, respectively) (Figure 2). In the training groups, heart contraction and relaxation velocities (± dp/dt max) were higher compared to the CTL group. The + dp/dt max increased in the HIIT + ISO and LIIT + ISO groups compared to the CTL group ($P < 0.001$, $P < 0.004$, and $P < 0.001$, respectively). However, + dp/dt max decreased in the ISO group compared to the control group ($P < 0.001$) (Figure 3). The -dp/dt max decreased in all groups compared to the CTL group (Figure 3).

Figure 1. Plasma Levels of Troponin I in Different Animal Groups



Values are means ± SEM; all groups are significantly different to the CTL group (†$P < 0.05$); •$P < 0.05$ versus the ISO group.

Table 3 shows the effects of low- and high-intensity training on myocardial tissue injuries. In the HIIT + ISO group, the rate of tissue damage was less in comparison to the LIIT + ISO and control groups; thus, high-intensity training induced a higher tolerance against ischemia (Table 3).

5. Discussion

With regard to I/R resulting from physical activity and its protective effects, the present study was performed to investigate the tolerance for myocardial ISO-induced ischemia following different training intensities with a sim-

Table 2. Hemodynamic Parameters and Ventricular Function of the Various Groups[a]

Groups/Variable	Ps, (mmHg)	Pd, (mmHg)	HR, (Beats/min)	MAP, (mmHg)	LVSP, (mmHg)	LVEDP, (mmHg)
CTL	112.3 ± 1.9	81.6 ± 1.7	345 ± 13.2	94 ± 3.01	127 ± 5[b]	3.6 ± 8[b]
ISO	99.8 ± 5.9	84.2 ± 4.7	381 ± 19.2	89.47 ± 4.9	102 ± 8[b,c]	14 ± 3[b,c]
HIIT + ISO	102.2 ± 6.2	74.2 ± 6.7	381 ± 12.6	82.82 ± 6.3	119 ± 5[b]	5 ± 1[b]
LIIT+ISO	106.6 ± 2.9	78.02 ± 3.7	410 ± 11.1	410 ± 11.1	114 ± 9[b,c]	8 ± 1[b,c]

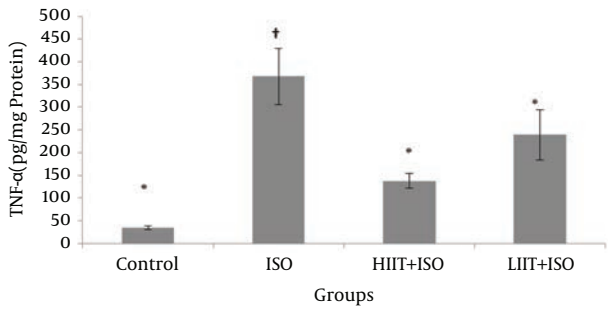
Abbreviations: CTL, control; ISO, isoproterenol; HIIT + ISO, high-intensity interval training + ISO; LIIT + ISO, low intensity interval training + ISO; Ps, systolic pressure; Pd, diastolic pressure; MAP, mean arterial pressure; HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure.
[a]Values are expressed as mean ± SEM.
[b]P < 0.05 versus CTL group.
[c]P < 0.05 versus HIIT + ISO group.

Table 3. Number of Animals Showing Different Pathology Scores in Various Groups

Group	Myocardial Lesion Score						
	N	0	1	2	3	4	Mean Rank
Control	8	8	-	-	-	-	4.50
ISO	8	-	-	-	3	5	26.38[a]
LIIT + ISO	8	-	-	2	4	2	21.88[a]
HIIT + ISO	8	-	2	6	-	-	13.25[a,b,c]

Abbreviations: CTL, control; ISO, isoproterenol; LIIT + ISO, low-intensity interval training + ISO; HIIT + ISO, high intensity interval training + ISO.
[a]P < 0.01 compared to the CTL group.
[b]P < 0.01 compared to the LIIT group.
[c]P < 0.001 compared to the ISO group.

Figure 2. Tissue TNF-α Levels in Different Groups

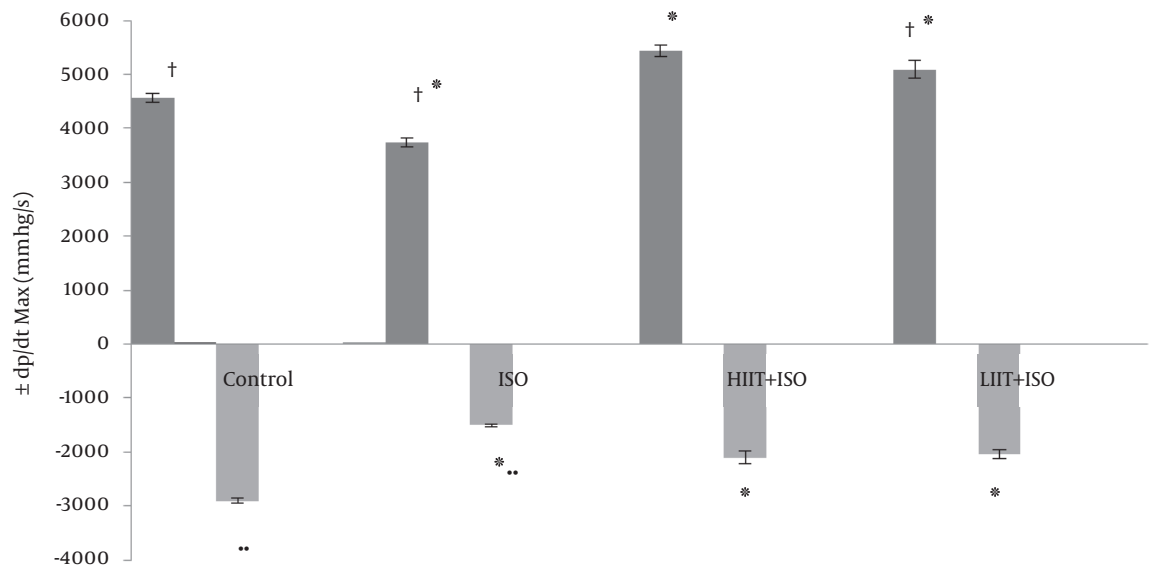


Values are means ± SEM; *P < 0.05 versus the ISO group; †P < 0.05 versus the HIIT + ISO group.

ilar work load. The two trained groups showed no differences in hemodynamic parameters, including Ps, Pd, MAP, and HR. However, in the trained groups compared to the control group, the heart function parameters (+ dp/dt max and dp/dt max) were better preserved.

Regular exercise, with its ischemia preconditioning effects, is one of the most effective protective factors against cellular death. I/R-related infarction and stunning (31) can cause heart remodeling (32). Exercise also alters cardiac extrinsic modulation more directly, and it improves the intrinsic pump capacity of the heart. Exercise, especially high-intensity training, causes better preservation of the intrinsic pump capacity through changes in the myocardium (32). According to Shannane E. Gormley's study (2008), high-intensity training compared to low-intensity training, without any effect on BP and HR of the resting condition, significantly increases VO_2 max, which is one of the most important indicators of physical fitness and cardiorespiratory function based on the Fick equation (33). Similarly, we observed that high-intensity interval training, without any significant changes in blood pressure and heart rate, increased the heart functional contractility (ventricular + dp/dt max). This emphasizes the protective effects of training based on its intensity. Michelson et al. (2012) (34) exposed the subjects to I/R and measured the heart tissue lesion size by a computer and scanner. They ultimately measured the ratio of ischemia size to the size of the at-risk region and concluded that high-intensity exercise decreases lesion sizes from 60% to 35%, similar to the histopathologic findings of the present study. In fact, high-intensity exercise releases endorphins, which activate the reperfusion injury salvage kinase (RISK), a potent media-

Figure 3. Maximum Contraction (+ dp/dt Max) and Relaxation (-dp/dt Max) Velocities in Different Animal Groups

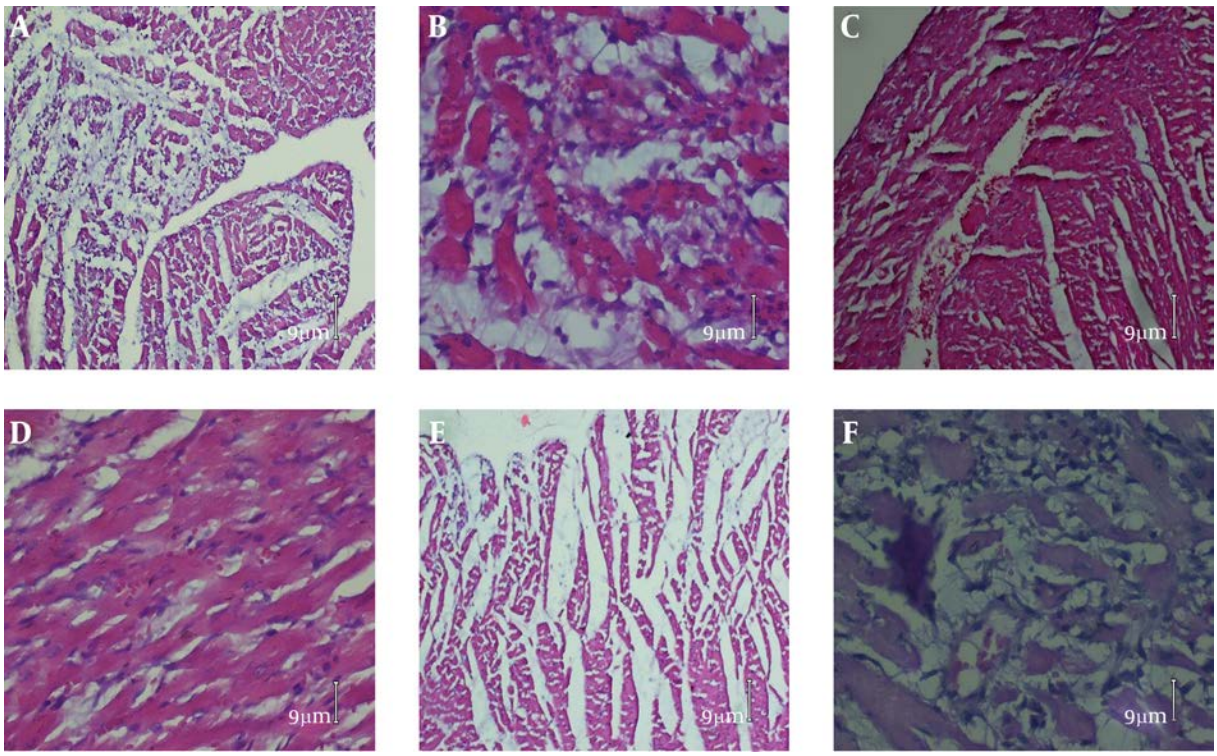


Values are means ± SEM; + dp/dt max: maximum velocity of heart contractions over time; -dp/dt max: maximum velocity of heart relaxations over time; *P < 0.05 versus the CTL group; †P < 0.05 versus the HIIT+ISO group; and ••P < 0.05 versus the LIIT + ISO.

tor of cardioprotection (35). Physiological adaptations (± dp/dt max alterations) might be attributed to the role of sarcolemmal K^+ ATP channels. Current studies have shown that sarcolemmal K^+ ATP channels are a potential mechanism in the protection against I/R resulting from exercise. Thus, the opening of sarcolemmal K+ATP channels accelerates cardiomyocyte depolarization through increasing the rate of K^+ exit and decreasing the action potential duration (36). Considering the value of this study, the duration of the training period (16 weeks) and the performance of regular exercise can justify these mentioned adaptations.

In the present study, the HIIT group showed lower serum cTnI levels in response to ISO administration compared to the CTL group. Based on previous studies, the cTnI levels show the intensity of heart tissue injury (20); therefore, this confirms the protective effect of a high-intensity training protocol. In relation to the acute response to the exercise intensity, there are some reports concerning the elevation of serum cTnI in subjects involved in high-intensity training, such as endurance competitions (23) and playing professional football (24). High-intensity training results in elevations of particular cardiac indicators like serum cTnI, and the elevation of these indices follow long training sessions (37). With regard to the higher stimulation/adaptation in high intensity exercise training, we observed higher adaptations and lower injury indices in the HIIT group, as probably in (37, 38). Although, currently, the training-induced heart protective mechanisms are still topics of discussion. However, it seems that in each session, higher intensity training stimulation and adaptation occurs. Training, as one of the factors of I/R (39, 40), has the potential to stimulate and produce free radicals, calcium overloading, protease activation (e.g., calpain), and the alteration of membrane lipids. Regular continuous exercise causes an adaptation and increase of myocardial tolerance against I/R (41, 42). Adaptation mechanisms against I/R, resulting from regular training, include the development of collateral vessels, thermal shock, and improvements and increases in antioxidant capacity. Antioxidants are one of the potent protective mechanisms against free radicals (42). Studies have shown that with long-term exercise, the elevation of antioxidant enzymes such as SOD, and the decrease of calpain activity, have important roles in heart protection following exercise (43, 44). According to previous studies, training decreases calpain activity and protects the heart in a manner similar to calpain pharmacologic inhibitors (43). Levine et al. (45) first described the elevated TNF-α levels in severe chronic heart failure. Moreover, following experimental myocardial infarction and ischemia in isolated cardiac myocytes and fibroblasts, TNF-α expression was up-regulated (46-49). These data demonstrate that the heart has an innate

Figure 4. Histopathological Examination of Heart Tissue in Various Groups



a, An ISO heart showing edema and necrosis of muscular fibers in the sub-endocardial region and acute myocardial thickness; b, An ISO heart showing degeneration and necrosis of muscular fibers and extensive infiltration of defense cells with the exit of defense cells from vessel walls and entering the edematous stroma; c, HIIT + ISO heart tissue showing mild intramuscular edema without degenerative necrosis with edema alterations in the sub-endocardial region that is more in the papillary muscles and less in the myocardium depth; d, HIIT + ISO tissue showing a small clot in an intramuscular vessel; e, LIIT + ISO tissue showing moderate and multi-focal edema and an infiltration of defense cells; f, LIIT + ISO tissue showing sub-endocardial edema associated with degenerative alterations and necrosis of tissues associated with degeneration and focal infiltration of defense cells (neutrophil and monocyte).

capacity to produce TNF-α in response to diverse pathophysiological stimuli like exercise. Moreover, Richard P. Sloan et al. (50) demonstrated that a 12 week aerobic training program significantly decreases TNF-α production in the whole blood in sedentary young adults, but this effect was seen only in the high-intensity training group, findings similar to the present study.

In conclusion, the regular exercise training programs in this study show protective effects in ISO induced myocardial infarction based on various biochemical and functional parameters. However, the high- and low-intensity training was beneficial. It can be said that regular high intensity interval training, compared to low-intensity interval training, has a higher cardioprotective potential. However, supporting the intensity of exercise is required for adaptation to occur when observing the beneficial effects. The literature suggests that optimizing exercise intensity is appropriate according to the physiological responses to exercises in all of the organs, and the HIIT has the tendency to induce superior positive effects, compared to LIIT, on cardiorespiratory fitness (CRF), traditional CVD risk factors, and biomarkers associated with vascular functions (51). To make a more definitive conclusion in this regard, a more thorough study is needed. The maximum exploitation of physical activity for health and fitness purposes is the result of choosing suitable exercise intensity.

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Footnote

Authors' Contribution: Hamid Marefati and Hamid Najafipour: studied the concept and design; Beydolah Shahouzehi, Soheil Aminizadeh, Siyavash Joukar, and Shahriar Dabiri: performed the data acquisition; Siyavash Joukar and Soheil Aminizadeh: performed the analysis and interpretation of the data; Hamid Marefati and Hamid Najafipour: drafted the manuscript; Hamid Marefati and Hamid Najafipour: performed the critical revision of the manuscript for important intellectual content; Siyavash Joukar and Soheil Aminizadeh: performed the statistical analysis; Hamid Marefati and Hamid Najafipour: took care of the administrative, technical, and material support; Hamid Marefati and Hamid Najafipour: supervised the overall study.

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