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# Comparison of the Effects of Different Training Intensities on Resistance to Induced Cardiac Ischemia in Male Adult Rats

Soheil Aminizadeh[1,3] , Hamid Marefati[2,3]*, Hamid Najafipour[3,4], Beydolah Shahouzehi[5]

[1]*M.Sc of Department Exercise Physiology, Faculty of Physical Education and Sport Sciences, Shahid Bahonar University of Kerman, Kerman, Iran*
[2]*Department of Exercise Physiology, Faculty of Physical Education and Sport Sciences, Shahid Bahonar University of Kerman, Kerman, Iran*
[3]*Physiology Research Center, Kerman University of Medical Sciences, Kerman, Iran*
[4]*Department of Physiology and Pharmacology, Afzalipour Medical Faculty, Kerman University of Medical Sciences, Kerman, Iran*
[5]*M.Sc of Clinical Biochemistry, Physiology Research Center, Kerman University of Medical Sciences, Kerman, Iran*

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## Abstract

**Purpose:** Myocardial infarction is one the main reasons of mortality in the world. One of the most important objectives of exercise training is to enhance stress tolerance and prevent heart attacks. Further studies are still needed on this type of prescribed exercise and heart condition improvement through exercise training (exercise preconditioning). The aim of this study was to compare the effects of two different exercise intensities (low and high) on the resistance rate in induced cardiac ischemia in male, adult rats based on performance (carotid ±dp/dt as an indicator of ventricular contraction and relaxation rate) and chemical factors of heart.

**Material and methods:** Twenty four male, adult, Wistar rats were randomly divided into three groups (n=8): control (C), low intensity training (LIT) and high intensity training (HIT). At the end of week 14 of the training, all rats received isoproterenol (85mg/kg) through ip injection for 2 consecutive days, then performance factors (HR, DBP, SBP, MAP, RPP, carotid ±dp/dt) and Chemical indicator of tissue damage (tissue CTnI) were measured.

**Results:** Functional factors including SBP (Systolic Blood Pressure), DBP (Diastolic Blood Pressure), HR (Heart Rate), MAP (Mean Arterial Pressure), and RPP (Rate Presure Product) in the exercise groups were not statistically different from the control group, but carotid ±dp/dt levels in high and low-intensity exercise groups were significantly higher as compared to the control group ($p<0.05$).Tissue Cardiac troponin I levels in high-intensity exercise group was significantly higher as compared to the control group ($p<0.05$).

**Discussion and Conclusion**: Our results showed that high-intensity exercise has protective effects against heart damage caused by injection of isoproterenol.

**Keywords:** Exercise intensity, Isoproterenol, Heart ischemia

## Introduction

Coronary artery disease and myocardial infarction are the most common reasons of mortality in many of the industrial countries and cause considerable inability and reduction of productivity of manpower [1,2]. Considering the effect of exercise preconditioning resulting in repetition of cardiac Ischemia/reperfusion, human and animal studies have shown supportive role of regular exercise in cardiac function [3,4,5,6]. But regarding the results of activity on cardiac function, different intensities and training protocols have shown different endogenous responses against heart deconditioning and have attracted many researchers.

Libonati et al [7] have showed that speed running (75m/min with 15% slope) improved resistance against ischemia in Longendorf system whereas distance running with low intensity (20m/min) did not show this positive effect.

It has been reported that there was an increase in serum cardiac troponin I in athletes who were training for distance running competitions [8,9] and also in professional football players and that this increase was greater than that of the control groups [10]. Swimming training for 60 minutes, two times a day increased –dp/dt during ischemia [11,12].

* Corresponding author E-mail:
marefati.h@uk.ac.ir

Korge et al [13] stated that medium intensity training was more efficient against ischemia as compared to high and low intensities. In another study Ahmadiasl et al [14] studied the effects of two short- and long-term resistant training protocols (70% 1RM), and observed an increasing resistance against ischemia considering coronary flow and ±dp/dt rised in response to strength training. They stated differences in training protocols as one of the reasons for possible contradictory findings (intensity, duration, kind). So it is important to offer a model that provides an improvement in cardiovascular function. Considering different responses and adjustments to different training protocols, the present study aimed to determine the optimal training intensity that could best improve cardiac function against induced ischemia.

## Material and Methods

***Drugs and chemical materials*:** Isoproterenol hydrochloride was supplied from Sigma-Aldrich Co, and thiopental sodium (Austria), cardiac troponin I ELISA kit (Kamiya Biomedical) and TNF-alpha ELISA kit (eBioscience) were also used.

***Animals*:** 24, three-month aged, Wistar, male rats with 200±50 g of weight were purchased from center of producing and keeping experimental animals in Kerman Medical University. Animals were kept in polyethylene cages, four in each cage (Tajhizgostar Co). food and water were provided ad lb. Rats were kept in room temperature ($22\pm2^0$c), in a twelve-hour darkness-light cycle. Animals were randomly divided into three groups (n=8): control-C, low intensity training(50%-55% VO2 max), and high intensity training( 90%-95% VO2 max).

**Training protocol:** Rats were trained for 14 weeks on a French treadmill after 2 weeks of familiarization during which they became familiar with the treadmill and tried to walk on it. This process started with 10 m/min for 10 minutes and after two weeks, reached 15 m/min in 30 minutes. The rats were trained with two intensities of HIT(42m/min) and LIT(24m/min) as an interval in the third week (3 minutes of warm up, 2 minutes of rest between sets, and two minutes of cooling down at the end of the protocol) (Tables 1 and 2) [15].

**Table 1:** High intensity training protocol

| weeks | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Speed(m/min) | 32.8 | 34 | 35 | 35.5 | 36 | 37 | 37 | 37.5 | 37.5 | 38 | 38 | 38 | 40 | 42 |
| Time(min) | 5 | 5 | 5 | 5 | 5 | 5.5 | 6 | 6 | 6.5 | 7 | 7 | 7.5 | 7.5 | 7.5 |

**Table 2:** Low intensity training protocol

| weeks | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Speed(m/min) | 18 | 18.7 | 20 | 21.4 | 22 | 22.5 | 23 | 23.5 | 23.5 | 24 | 24 | 24 | 24 | 24 |
| Time(min) | 9 | 9 | 8.75 | 8.1 | 8.1 | 9 | 9.6 | 9.5 | 10.3 | 11 | 11 | 11.8 | 12.5 | 13 |

***Empirical myocardial infarction, sampling and recording:***

Isoproterenol (85 mg/kg) was dissolved in normal saline and was injected intra peritoneally (ip) during two days within 24 hours in order to create empirical myocardial infarction. After 24 hours, rats were anesthetized by ip injection of 50 mg/kg thiopental sodium. After deep anesthesia, their tracheas were channeled and they were allowed to breathe spontaneously during experiment. Heart rate and arterial blood pressure entered carotid with a canal filled by heparin saline (15unit/ml) and they were attached to physiographic machine and pressure transducer (Beckman R612 USA). Mean arterial pressure (MAP) was calculated as follows:

(MAP = DAP + (SAP-DAP) / 3)

and myocardial oxygen demand was obtained with the following formula:

[(MAP * heart rate) /1000] [34].

After recording the functional factors of the heart for 20 minutes, heart tissue was removed for biochemical experiments [16].

***Preparation and homogenization of tissue***

After anesthetizing rats and recording functional factors, heart tissue was rapidly removed and washed in cold normal saline and was placed

quickly in liquid nitrogen. Heart tissue was homogenized in cold buffer and protein concentration was measured by Lowry method [33] then TNF-alpha and CTnI levels were assessed using the appropriate ELISA kits.

### *Statistical analysis*

Data were expressed as mean±SEM and were analyzed using SPSS software (version 20). In order to analyze data we used one way ANOVA followed by post hoc Tukey. P<0.05 was considered as statistically significant.

## Results

Isoproterenol damaged heart tissue in all the study groups through significantly decreasing cardiac tissue troponin I. Results showed that cardiac tissue troponin I was significantly higher in high intensity training group as compared to the control group. Although low intensity training group showed more cardiac tissue troponin I as compared to the control group, this difference was not significant between the two groups (p>0.05). Systolic Blood Pressure, HR, MAP, rate pressure product (RPP) and Diastolic Blood Pressure were not significantly different between the exercise and control groups (p>0.05) (Table 3).

**Table 3:** Functional and biochemical factors in the study groups

| Groups | Rate pressure product(RPP) mmHg | Mean arterial pressure(MAP) mmHg | Heart rate(HR) Beats/min | Diastolic Blood Pressure(DBP) mmHg | Systolic blood pressure(SBP) mmHg | +dp/dt Carotid | -dp/dt Carotid | Tissue CTnI Ng/mg |
|---|---|---|---|---|---|---|---|---|
| **Control** | 34/24±3/01 | 89/47±4/98 | 381±19/27 | 84/27±4/71 | 99/88±5/94 | 474±71 | -204±28 | 28/73±1/92 |
| **High intensity** | 31/61±2/75 | 82/82±6/32 | 381±12/66 | 74/21±6/75 | 102/20±6/27 | 1401±98* | -914±70* | 49/51±5/67* |
| **Low intensity** | 35/98±1/94 | 87/55±3/37 | 410±11/18 | 78/02±3/77 | 106/60±2/30 | 1274±78* | -767±92* | 39/00±2/00 |

Results are expressed as mean±SEM.
Significant points are marked with *.

## Discussion

According to Ischemia/reperfusion properties resulted from physical activity and its protective effects, we examined the resistance rate response to isoproterenol-induced cardiac ischemia at different exercise intensities with the same work load.

There was no difference regarding functional factors (HR, MAP, RPP, SBP and DBP) between the groups. Whereas there was a better cardiac functional index (Carotid ±dp/dt max) in the training groups as compared to the control group. Training may change extrinsic modulation and improve the intrinsic pumping capacity of the heart. intensive training provides better conservation of the intrinsic pump capacity by affecting myocardium [17]. In fact regular training accounted for one of the protective motivations against necrosis and infarction resulted from I/R and Stunning, because of the Ischemia Preconditioning potential that can result in cardiac remodeling [18,19]. Shannan et al (2008) showed that compared to lower intensity training, high intensity training could significantly increase VO2 max, without affecting rest state HR and BP. VO2 max is considered as one of the most important physical fitness factors and according to Fick's equation, is a cardiac-pulmonary function index [20]. In the present study also cardiac functional index (Carotid ±dp/dt max) increased without affecting rest state HR and BP in high intensity training group which could probably be an emphasis on I/R effects proportional to intensity. John C (2006) demonstrated that some days of intermediate intensity training did not result in cardiac arteries remodeling and cardiac architecture, but showed beneficial effects on biochemical factors which was also an important finding of the present study that included regular training principle observance for 4 month[21].

In this study high intensity training group showed lower serum CTnI levels in response to injected Isoproterenol as compared to the control group. In fact Isoproterenol produces free radicals through beta adrenoceptor mechanism and affects cellular metabolism that causes cytotoxic necrosis of cardiac muscle[22]. According to the conducted researches cardiac troponin I levels is an indicator of the cardiac injury severity[23]. As mentioned earlier CTnI had negative correlation with training intensity, so it could be proved that the exercise protocols have cardio protective properties. However, mechanisms responsible for exercise-

induced myocardial protection are considered as a controversial topic. Exercise as a I/R factor has the potentiality to stimulate production of free radicals, calcium overload, activation of proteases (calpein) and change the membrane's lipid. On the other hand, regular exercise causes myocardial adaptation and increased tolerance against I/R [24, 25, 26, 27, 28]. Exercise-induced adaptation mechanisms against I/R include development of collateral vessels, heat shock proteins, and recovery and increase in antioxidant capacity. Antioxidants are introduced as one of the strongest protective mechanisms against free radicals [28]. Evidence suggests that long term exercise increases antioxidant enzymes such as manganese superoxide dismutase (MnSOD) and decreases calpein activity that plays an important role in protecting heart from damage followed by exercise [29, 30]. Exercise adjusts antioxidants and improves survival courses of cells and improves their ability to overcome ROS and calcium increases. Reduction of calcium due to exercise needs production of endothelial NOS;improved NOS is forced to act as PKC4[31] Other studies such as Pafenberger and Hall reported that incidence of myocardial infarction and appearance of other cardiac ischemia was less in ship porters, who consume more energy in their works[32].Studies have showed that exercise reduces calpein activity and provides cardiac protection such as calpein pharmacological inhibitors [29].

The results of this study showed protective effects of high-intensity exercise on isoproterenol-induced myocardial infarction that may be resulted from increased antioxidants levels in the heart tissue and prevention of membrane severe damage.

## Conclusion

Results of this study revealed protective effects of high intensity training on Isoproternol-induced myocardial infarction which maybe the result of antioxidant increase in cardiac tissue that prevents severe damage of membrane.

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