











Biomarker responses of cardiac oxidative stress to high intensity interval training in rats

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Abstract - Aim: The present study aimed to verify the cardiac oxidative stress biomarker responses to high-intensity interval training (HIIT) in rats. **Methods:** Sixteen male Wistar rats weighing 250 to 300 g were equally divided into two groups (8 animals/group): sedentary control (SC) and trained group (HIIT). The exercise protocol consisted of high-intensity swimming (14% of body weight, 20 s of activity with 10 s of pause performed 14 times) which was performed for 12 consecutive days. **Results:** The cardiac tissue concentrations of malondialdehyde and carbonylated proteins showed no significant changes; on the other hand, hydroperoxide levels were higher in the HIIT group than in the SC group. The activities of superoxide dismutase, catalase, and glutathione peroxidase enzymes and the levels of reduced glutathione and sulfhydryl remained unchanged. **Conclusion:** It is possible to conclude that short-term high-intensity interval training induces changes in the cardiac oxidative stress biomarker but with no effect on the antioxidant enzymes.

Keywords: physical training, lipid peroxidation, heart, HIIT, swimming.

Introduction

High-intensity interval training (HIIT) is characterized by brief and repeated episodes of vigorous activity (approximately 85%-100% of the maximal oxygen uptake [VO_{2max}]) followed by short periods of passive or active rest with exercises¹⁻³. HIIT is considered a promising method for the reduction of cardiometabolic risk factors^{4,5}. Oxidative stress is defined as the imbalance between reactive oxygen species ROS production and intracellular antioxidant defense capacity⁶⁻¹⁰. The production and exacerbated release of ROS generated by physical exercise can disrupt intracellular redox homeostasis¹¹, which can lead to protein oxidation, lipid peroxidation, and DNA damage¹² as well as lesions in cardiac cells¹⁰. As a consequence, cellular responses to significant unrepaired damage can lead to apoptosis or senescence, contributing to the onset of cardiovascular diseases and, in some cases, metabolic syndrome¹³⁻²⁰.

There is already evidence that HIIT can induce less membrane peroxidation, and greater competence in the antioxidant system, reduction in central mediators of necroptosis induced by myocardial infarction, in addition

to cardioprotection against ischemia-reperfusion injury²¹ and the expression of cardioprotective proteins in a similar way to continuous exercise²². In this sense, the control of the training load is fundamental for the achievement of your specific objectives, among them, the improvement of performance. Therefore, the imbalance between volume, intensity, and density in the training session, an increase in the concentration of ROS is visible, which can lead to oxidative stress. Thus, this study verified the responses of cardiac oxidative stress biomarkers to training in rats. The hypothesis is that HIIT can prevent the occurrence of exacerbated oxidative stress in the cardiac tissue.

Methods

Animals and experimental groups

Sixteen male Wistar rats (*Rattus norvegicus*) weighing 250 to 300 g and 60 days of age at the beginning of the experiment were kept under normal environmental conditions with a temperature of $24 \text{ }^{\circ}\text{C} \pm 2 \text{ }^{\circ}\text{C}$ and a light-dark cycle of 12 h, with free access to filtered water and standard commercial diet (Labina, Purina[®]). The animals were

randomly allocated to two experimental groups (n = 8/group), sedentary control group (CS), and trained group (HIIT), and kept in collective cages (4 animals/cage). All procedures were approved by the Ethics Committee for Animal Use at the Federal University of Sergipe (Process number 15/2017) and followed the Guidelines of the Brazilian College of Animal Experiments (COBEA).

Adaptation to water

The animals were acclimated and adapted to the liquid environment at a temperature of 25 °C ± 1 °C in a cylindrical tank of 80 cm depth and 80 cm diameter¹⁷. During the first week, only a 10 min adaptation was performed in the water at a depth of 10 cm. In the subsequent two weeks, the animals had a lead overload (small bags of cotton cloth and Velcro[®]) attached to the chest for 10 days; the overloads used were equivalent to 0%, 1%, and 2% of the body weight, and each animal was subjected to 10 min of swimming exercise with 30 s of swimming and 30 s of rest between the series, totaling 10 series. It is important to note that the adaptation period was not capable of inducing possible physiological changes due to the low intensities used^{17,18}.

Physical training

Physical training was performed according to a protocol adapted from the study by Terada et al.²³. The animals, which were submitted to overloads equivalent to 14% of body weight, according to the authors this load is sufficient to superimpose an intensity of 80% of the animals' VO₂max, also considered to be of high intensity^{24,25}. The rats performed a 20 s swimming session, 14 times. Between each repetition, the animals were allowed a rest of 10 s. All rats swam in tanks at a water depth of 60 cm, for 12 continuous days.

Euthanasia and preparation of tissues

Twenty-four hours after the end of the last physical training session, the animals were anesthetized with ketamine/xylazine (75 mg/kg + 10 mg/kg i.p) followed by euthanasia via bleeding under anesthesia. Then, the heart was removed, washed three times with 1.15% potassium chloride (KCl) solution, dried, weighed, and stored in a biofreezer at -80° for further analyses of oxidative stress biomarkers.

Biochemical analyses

Lipoperoxidation products were measured in two ways: a) concentration of lipid hydroperoxides (HPx) by the oxidation technique of xylenol orange, in which oxidation of ferrous ion (Fe²⁺) into ferric ion (Fe³⁺) occurs under acidic conditions; b) concentration of thiobarbituric acid reactive substances²⁶. The concentration of carbonylated proteins in assays was determined by the technique of Lowry et al.²⁷, which quantified the concentrations of

proteins in the samples by comparison with a standard curve obtained from bovine serum albumin at different concentrations.

The activity of the superoxide dismutase enzyme (SOD) was determined by the capacity of the tissue enzyme to dismutase superoxide anions derived from the pyrogallol autoxidation and their reaction, reducing bromide 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium and forming formazan crystals²⁸. Catalase (CAT) activity was determined by the hydrogen peroxide (H₂O₂) degradation rate according to the standard protocol previously described by Nelson and Kiesow²⁹. The activity of glutathione peroxidase enzyme (GPx) was evaluated by NADPH oxidation, as described by Paglia and Valentine³⁰. The activity of the glutathione reductase enzyme was assessed according to the method of Carlberg and Mannervik³¹. The determination of sulfhydryl groups was performed by the reaction between 5'5-dithio-bis-2-nitrobenzoic acid and free sulfhydryl of the cysteine side chain³².

Statistical analyses

Data are expressed as mean ± standard deviation. Data normality was tested by the Shapiro-Wilk test. Non-paired Student's t-tests were used to evaluate the differences between the groups. P-values < 0.05 were considered statistically significant. For all of these procedures, the statistical software GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA) was used.

Results

Regarding the cardiac oxidative stress biomarkers (Table 1), there was a significant increase for the HIIT group in the HPx levels when compared to the CS group. Regarding antioxidant defense, there was no change in any of the levels of MDA and PC parameters evaluated between the groups (Table 2).

Discussion

The present study verified the effect of HIIT on the cardiac oxidative stress responses in rats. The study showed that consecutive HIIT sessions caused an increase in HPx levels.

Table 1 - Effects of high intensity interval training for 12 days on oxidative stress markers hydroperoxides (HPX), malondialdehyde (MDA), and carbonylated proteins (PC) in cardiac tissues from rats.

	CS (n = 08)	HIIT (n=08)	p (value)
HPX (µmL)	3.288 ± 0.2594	5.988 ± 0.5595	0.0006
MDA (nmol/mL)	8.386 ± 0.5837	8.694 ± 0.6250	0.07
PC (nmol/mL)	208.2 ± 10.62	217.0 ± 9.377	0.85

Note: CS = sedentary control group and HIIT = trained group. Data are presented as mean ± SEM. Student's t-test. (p<0.05).

Table 2 - Effects of high intensity interval training for 12 days on enzymatic antioxidant activity superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR), and non-enzymatic total sulfhydryl (SH) in cardiac tissues from rats.

	CS (n = 08)	HIIT (n=08)	p (value)
SOD (U/mg protein)	0.1265 ± 0.01760	0.1661 ± 0.03506	0.33
CAT (U/mg protein)	0.02038 ± 0.003845	0.03225 ± 0.005864	0.11
GPx (U/mg protein)	0.8525 ± 0.1435	0.83 ± 0.127	0.91
GR (µmol)	1.819 ± 0.632	1.378 ± 0.2635	0.53
SH (nmol/mL)	208.2 ± 10.62	217.0 ± 9.37	0.54

Note: CS = sedentary control group and HIIT = trained group. Data are presented as mean ± SEM. Student's t-test. (p < 0.05).

Swimming HIIT was performed using a 14% load. The intensity of the training was defined by the load test, swimming with a load above 6% of the bodyweight of the animal is considered to be high-intensity exercise¹⁹. Variations of intensity, type, and duration of exercise directly influence the production of ROS, and consequently, the oxidative damage caused by it. High-intensity exercise has been shown to cause higher lipid oxidation in the liver, muscle, and blood³³. During the contractile activity, an intracellular increase of O₂[•], hydrogen peroxide (H₂O₂), and nitric oxide occur. It is suggested that the action of the enzyme NADPH oxidase in the plasma membrane, cellular cytosol, and sarcoplasmic reticulum in the skeletal and cardiac muscle and also in the localized transverse tubules of the skeletal muscle is the main source of free radical production associated with physical exercise⁹.

Since HPx is considered a specific and direct biomarker of lipid peroxidation, the higher HPx concentrations in the HIIT group compared SC group in the present study show that this type of training, associated with short recovery periods, can generate adaptations in cardiac tissue^{34,35} as well as cause structural remodeling of the cell membrane and its lipoproteins²³, both resulting from the momentary hypoxia induced by HIIT. Casuso et al.³⁶ found a decrease in the plasma HPx levels in swimmers subjected to hypoxia compared with those maintained under normal conditions. Some studies indicate that the cardioprotective effects of moderate to severe exercise can be attributed to a decrease in vascular inflammation and oxidative damage⁴, confirming previous data on blood plasma³⁷. Increases in serum levels of aspartate aminotransferase and alanine aminotransferase enzymes following damage to tissues like the heart increases the levels of some markers, such as malondialdehyde (MDA; an authentic index of oxidative stress), and decreases the levels of antioxidant enzymes such as SOD, GPx, and CAT³⁸. In the present study, there were no significant alterations in MDA levels in the cardiac tissue of rats. On the other hand, Freitas et al.³⁹ demonstrated a reduction of this lipoperoxidation biomarker in rats submitted to 36 running sessions on a treadmill, unlike the present study which used the swimming exercise. Thus, we cannot rule out that the protocol-dependent design may have generated divergent results among studies. On the other hand, in

a study involving humans, Tauler et al.⁴⁰ measured both plasma MDA and lymphocyte protein carbonyl levels following a mountain stage cycling protocol. They reported a significant increase in both indices of oxidative stress.

Rosa-Lima et al.¹² emphasized that protein oxidation can cause cell death, and carbonylated proteins are indirect markers of protein damage⁴¹. Oxidative damage can have a devastating effect on the structure and activity of proteins and can even lead to cell death. Amino acids containing cysteine and methionine are particularly susceptible to ROS and reactive chlorine species, which can damage proteins⁴². In the present study, there were no significant changes in the carbonylated proteins in any of the experimental groups, denoting that the HIIT protocol used did not promote oxidative damage in the cardiac tissue. HIIT has been an important protocol of signaling to a multitude of target cells allowing aerobic adaptations during the short-term, further than the traditional endurance training⁴³. Some studies have reported that endurance training in rats may attenuate the natural loss of protein but not increase the aerobic capacity in comparison with the baseline⁴⁴.

Exercise stimulates the production of ROS in tissues and blood due to large increases in oxygen uptake, while ROS are formed during physical stress, the antioxidant system improves the endogenous enzymes⁴⁵. Azizbeigi et al.⁴⁶ reported that high-intensity exercise strengthens the defensive system of erythrocytes against free radical damage. The results obtained in the present study did not indicate changes in the cardiac tissue concentrations of sulfhydryl groups in response to HIIT as in the activity of antioxidant enzymes, corroborating the findings of de Araújo et al.⁴³ and Songstad et al.⁴⁷, who subjected rats to high-intensity training with water jumping and treadmill running, respectively. The antioxidant enzyme levels remained unchanged significantly in relation to control, showing an insignificant disturbance of ROS.

It is important to note that the present study investigated only the effects of HIIT on the cardiac oxidative stress markers in rats, with the possible limitation of the use of the swimming model for HIIT. Hence, it is suggested to use other types of ergometers such as water jumping or ladder climbing for comparison purposes.

Conclusion

Taken together, the results of the present study suggest that short-term HIIT induces changes in the cardiac oxidative stress biomarker responses without affecting the antioxidant enzymes analyzed.

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