



Trade Science Inc.

ISSN : 0974 - 7427

Volume 6 Issue 3

# BioCHEMISTRY

*An Indian Journal*

*Regular Paper*

BCAJI, 6(3), 2012 [88-93]

## Effect of different frequencies of endurance training on antioxidant defense in non-athletes after exhaustive exercise

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Received: 7<sup>th</sup> March, 2012 ; Accepted: 4<sup>th</sup> April, 2012

### ABSTRACT

**Objective:** We investigated the effects of endurance training with different frequencies on the blood antioxidant enzymes activities and indexes of lipid, protein and DNA damage after exhausted exercise. **Methods:** 30 non-athlete healthy subjects were divided into two experimental groups (A and B) and one control group (C), involving 10 people in each group. All groups underwent Bruce test. Then experimental groups were subjected to five-week endurance training with different frequencies. All groups underwent treadmill exercise for the second time 72 hour after last week. After each Bruce test, blood sampling was obtained from capital vein for biochemical measurements. **Results:** In groups A and B, antioxidant enzyme activities and the levels of oxidative damage biomarkers after endurance training were significantly higher than before training condition. After endurance training, higher enzyme activities were found in groups A, B, followed by C. The levels of oxidative damage biomarkers in group A and B after endurance training were significantly higher as compared with control group. The lowest levels of these indexes were related to control group. **Conclusions:** This study suggests that high and low training frequencies increases the antioxidant enzyme activities and the levels of oxidative damage biomarkers. However, increase in antioxidant enzyme activities is more remarkable in high frequency training.

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### KEYWORDS

Endurance training;  
Frequency;  
Antioxidative enzymes;  
Oxidative damage;  
Bruce test.

### INTRODUCTION

During normal mitochondrial metabolism, 2% to 5% of the consumed oxygen ( $VO_2$ ) may be convert to free radicals such as reactive oxygen species (ROS)<sup>[7]</sup>, which are involved in the pathology of many diseases<sup>[36]</sup> including diabetes, inflammation, cancer, cardiovascular

disorders, and process of ageing<sup>[25]</sup>. Exercise is associated with an increase in  $VO_2$  and results in increased production of free radical<sup>[7,19]</sup>. ROS can react with cellular components such as lipids, proteins, and DNA<sup>[36]</sup>, that leads to formation of oxidative stress biomarkers, including malondialdehyde (MDA), reactive carbonyl derivatives (RCD), and 8-hydroxy-2'-deoxyguanosine

(8-OHdG)<sup>[1,6]</sup>. Cells are protected from free radical damage by enzymatic and non-enzymatic antioxidant mechanisms<sup>[16,21]</sup>. The major antioxidant enzymes are superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT)<sup>[30]</sup>.

Free radical formation due to exercise is raised up as compared with rest levels. Therefore, antioxidant enzyme activities are increased subsequently<sup>[10,35]</sup>. Many studies have shown that improvement in antioxidant defense after endurance exercise is due to compatibility in antioxidant defense system<sup>[12,18,31]</sup>. Related Studies reported no change or decrease in antioxidant defense system after endurance exercises<sup>[4,11,23]</sup>. Most of these studies have used different protocols, resulted various consequences<sup>[3,24,34]</sup>. There is no standardized protocols that have been used in exercise training.

Difference in training programs is due to difference intensity, frequency and duration of exercise<sup>[31]</sup>. Due to importance of exercise program designing, it is necessary to know commodious training programs to gain ideal results, preventing expense, time and energy waste.

This study aimed to investigate whether different frequencies of training at the same intensity and duration affect the antioxidant system of the body.

## METHODS

### Subjects

Thirty healthy, non-athlete volunteers were randomly chosen in this study. They aged 20-25 years. The main indicators in this research were no smoking and alcohol, no intake of antioxidant supplements, no history of heart disease and respiratory failure, and no anti-inflammatory drugs. Subjects gave their informed written consent to participate in the study. They were divided into two experimental groups of ten people (groups A and B) and one control group of ten people (C). All groups underwent treadmill exercise testing using the Bruce protocol. Then group A and B were subjected to a five-week endurance training (group A; 20 minutes per day and 6 days per week, group B; 40 minutes per day and 3 days per week). These groups exercised at the same intensity (75% to 85% maximal heart rate) and their heart rate monitored with polar S810 heart rate monitor. Group C remained without exercise. 72 hours after last exercise session, all groups underwent

treadmill exercise for second time.

### Exercise treadmill test (Bruce test)

Bruce test was done by treadmill. It had seven steps and each step took 3 minutes. At the beginning, the slope of the treadmill was 10% and its rate was 2.7 km/h. In each 3 minutes, the slope increased 2% and the rate increased 1.47 times. It continued until the subject was unable to run<sup>[29]</sup>.

### Samples

After each Bruce test blood sampling was obtained from capital vein. Blood samples were withdrawn into heparinized tubes. Heparinized whole blood samples were immediately centrifuged at 3000 rpm in 4 °C for 10 minutes. The supernatant was aspirated and collected as serum and stored at -70 °C. To separate erythrocytes, the buffy coat was drained and the sediment was washed four times with 3 ml of 0.9% sodium chloride solution, and was centrifuged under the same conditions. Aliquots of the washed erythrocytes were lysed by freezing (-20°C) for 24 h and then they were used for measurements.

### Antioxidant enzymes assay

The SOD activity in erythrocytes was measured according to the method of Misra & Fridovich<sup>[20]</sup> on the basis of their ability to inhibit free radical chain oxidation in which O<sub>2</sub> was a chain-propagating radical and the autooxidation of epinephrine was included. Human erythrocyte SOD was used as a standard and the activity was expressed in Unit/g.Hb.

CAT activity was determined by monitoring the disappearance of H<sub>2</sub>O<sub>2</sub> at 240 nm. CAT activity was expressed as unit/mg.protein. One unit of enzyme was the amount necessary to decompose 1 μmol of H<sub>2</sub>O<sub>2</sub> per min at 25°C<sup>[5]</sup>.

GPX activity was measured by the method of Paglia & Valentine<sup>[22]</sup>. Heparinized whole blood (0.05 ml) was diluted with 1 ml diluting agent, incubated for 5 min, and then 1 ml of double strength Drabkin's reagent was added and mixed well. GPX catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of GSH reductase and nicotinamide adenine dinucleotide phosphate (NADPH), the oxidized GSH was immediately converted to the reduced form with the concomitant oxidation of NADPH to NADP<sup>+</sup>. The

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decrease in absorbance at 340 nm was measured by a spectrophotometer.

### Measurement of oxidative damage biomarkers

MDA concentration in plasma was measured by the high-performance liquid chromatography technique (model 4225; Unicam, LCD/Analytical) in which the MDA–thiobarbituric acid (TBA) adduct was separated<sup>[32]</sup>. Briefly, plasma lipoperoxides were hydrolyzed by boiling in diluted phosphoric acid. MDA was reacted with TBA to yield the MDA–TBA adduct. The protein-free extract was fractionated on a C18 column of octadecyl silicagel to separate the MDA–TBA adduct by elution with methanol/phosphate buffer and quantified by a spectrophotometer at 532 nm (model: crystal 200; Unicam LCD/Analytical Inc.).

RCD were detected by their reactivity with DNPH to form protein hydrazones and their amount evaluated at 370 nm and expressed in nmol/mg.protein<sup>[28]</sup>.

8-OHdG levels were measured essentially as described previously<sup>[8]</sup>. Briefly, an automated column switching LCEC method for 8-OHdG was based on the unique selectivity of the integral porous carbon column for purines.

Samples were injected onto a C8 column and the band containing 8-OHdG was then quantitatively trapped on a carbon column. The selectivity of the carbon column for 8-OHdG allowed elimination of interfering peaks by washing the column with a second mo-

bile phase and then eluting 8-OHdG to an analytical C18 column with an identical mobile phase containing adenosine to displace 8-OHdG. Detection with series colorimetric electrodes provides qualitative certainty for 8-OHdG peak by response ratios.

### Statistical analysis

Values were expressed as mean  $\pm$  SEM. The results were analyzed by one-way analysis of variance (ANOVA) followed by Paired-Samples T Test to evaluate the significance of the differences between groups using Statistical Package for Social Science (SPSS 16.0 for windows). A  $p < 0.05$  level of significance was used.

## RESULTS

Anthropometric characteristics of voluntary subjects are demonstrated in TABLE 1. Subjects were at the same range of age with mean of 22.5 to 22.6 years. There were no significant differences between groups in height, weight, and BMI as they were evaluated before and after endurance training. In groups A and B,  $VO_{2max}$  increased significantly after endurance training as compared to before endurance training ( $p < 0.05$ ), but there was no significant difference in control group ( $p > 0.05$ ). In addition, differences in  $VO_{2max}$  were not significant between groups A, B, and C before and after exercise ( $p > 0.05$ ).

TABLE 1 : Physical characteristic of studied groups before and after endurance training. Data are expressed as mean  $\pm$  SEM.

Groups		Age	Weight	Height	BMI	$VO_{2max}$ (ml/kg.min)
Group A (N = 10)	before	22.5 $\pm$ 2.2	71.91 $\pm$ 4.4	1.76 $\pm$ 3.3	23.21 $\pm$ 2.4	36.21 $\pm$ 2.3*
	after	22.5 $\pm$ 2.2	71.88 $\pm$ 4.5	1.76 $\pm$ 3.3	23.20 $\pm$ 1.6	40.47 $\pm$ 2.1
Group B (N = 10)	before	22.6 $\pm$ 2.2	68.16 $\pm$ 5.4	1.75 $\pm$ 4.4	22.25 $\pm$ 3.3	39.99 $\pm$ 2.4*
	after	22.6 $\pm$ 2.2	68.17 $\pm$ 5.2	1.75 $\pm$ 4.4	22.25 $\pm$ 3.2	44.89 $\pm$ 2.2
Group C (N = 10)	before	22.5 $\pm$ 2.4	63.9 $\pm$ 1.2	1.73 $\pm$ 5.4	21.35 $\pm$ 2.3	40.14 $\pm$ 3.7
	after	22.5 $\pm$ 2.4	63.9 $\pm$ 1.2	1.73 $\pm$ 5.4	21.35 $\pm$ 3.6	41.03 $\pm$ 3.7

\* Differed significantly with respect to after ( $p < 0.05$ ).

Antioxidant enzyme activities before and after endurance training in groups A, B, and C are presented in TABLE 2. In each studied group, SOD, GPX, and CAT activity elevated after five weeks. In groups A and B, which undergone endurance training, these enzymes activities after endurance training were significantly higher with respect to before training ( $p < 0.05$ ). However, in

group C, which had no training activity, SOD, GPX, and CAT did not differ significantly during the study time ( $p > 0.05$ ). SOD, GPX, and CAT activity of group A and B after endurance training were significantly higher as compared with control group ( $p < 0.05$ ), but there were not any significant difference before endurance training ( $p > 0.05$ ).

TABLE 2 : Antioxidant enzyme activities before and after endurance training in groups A, B, and C in exhaustive state.

Enzyme	Group	before endurance training	after endurance training
SOD (U/mg.pro)	Group A (N = 10)	1975.30 ± 68.90*	5471.50 ± 230.13 <sup>Ⓢ</sup>
	Group B (N = 10)	1943.10 ± 67.13*	3588.20 ± 157.26 <sup>†</sup>
	Group C (N = 10)	1925.10 ± 32.90	1984.00 ± 44.99
GPX (U/mg.pro)	Group A (N = 10)	85.60 ± 2.37*	189.80 ± 12.17 <sup>Ⓢ</sup>
	Group B (N = 10)	93.38 ± 3.19*	130.69 ± 6.61 <sup>†</sup>
	Group C (N = 10)	88.67 ± 3.39	93.34 ± 2.99
CAT (U/mg.pro)	Group A (N = 10)	766.00 ± 35.52*	1516.90 ± 101.58 <sup>Ⓢ</sup>
	Group B (N = 10)	736.10 ± 13.92*	1146.60 ± 33.30 <sup>†</sup>
	Group C (N = 10)	686.20 ± 21.22	722.70 ± 18.41

\* differed significantly as compared with enzyme activity after endurance training ( $p < 0.05$ ). <sup>Ⓢ</sup> differed significantly as compared with group B ( $p < 0.05$ ). <sup>†</sup> differed significantly as compared with group C ( $p < 0.05$ ).

TABLE 3 demonstrates the levels of oxidative damage biomarkers before and after endurance training in groups A, B, and C. Blood samples collected after five week endurance training revealed higher levels of MDA, RCD, and 8-OHdG in group A and B, and differences were meaningful between samples collected before and after endurance training ( $p < 0.05$ ). In group C, there were no significant differ-

ences for these three indexes after five week study ( $p > 0.05$ ). MDA, RCD, and 8-OHdG concentrations after endurance training differed significantly between groups A and C as well as between groups B and C ( $p < 0.05$ ), but they were not significant before endurance training ( $p > 0.05$ ). RCD concentration were higher in group A and B and then C but 8-OHdG level followed this order, B > A > C.

TABLE 3 : The levels of oxidative damage biomarkers before and after endurance training in groups A, B, and C in exhaustive state.

Biomarker	Group	before endurance training	after endurance training
MDA ( $\mu\text{mol/mg.pro}$ )	Group A (N = 10)	187.90 ± 5.50*	237.10 ± 11.57 <sup>†</sup>
	Group B (N = 10)	187.80 ± 5.06*	238.60 ± 6.81 <sup>†</sup>
	Group C (N = 10)	201.70 ± 6.08	212.20 ± 11.42
RCD (nmol/mg.pro)	Group A (N = 10)	131.20 ± 2.65*	201.10 ± 5.72 <sup>†</sup>
	Group B (N = 10)	128.50 ± 2.98*	189.40 ± 4.50 <sup>†</sup>
	Group C (N = 10)	129.20 ± 4.02	127.70 ± 5.34
8-OHdG (nmol/mg.pro)	Group A (N = 10)	32.53 ± 1.25*	40.73 ± 1.56 <sup>†</sup>
	Group B (N = 10)	33.14 ± 1.42*	44.57 ± 1.47 <sup>†</sup>
	Group C (N = 10)	32.41 ± 1.16	33.59 ± 1.32

\* differed significantly as compared with after endurance training ( $p < 0.05$ ). <sup>†</sup> differed significantly as compared with group C ( $p < 0.05$ ).

## DISCUSSION

In this research, we investigated the effects of endurance training with different frequencies on antioxidant enzyme activities and the levels of selective oxidative damage biomarkers after exhaustive exercise. No study reported the effect of endurance training frequency on adaptive responses in body to encounter with exhaustive conditions. However many

studies have investigated the effect of exercise on antioxidant enzyme activities<sup>[24,34]</sup>. Most of them reported increased antioxidant enzyme activities after exercise<sup>[9,14,33]</sup>. Our findings, in agreement with other studies, showed significant increase in antioxidant enzyme activities in trained groups with respect to control. ROS activate specific redox-sensitive transcription factors, such as AP-1 and NF- $\kappa$ B in the promoter regions of genes encoding SOD, GPX, and CAT<sup>[17]</sup>. The body increases the production of anti-

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oxidant enzymes as an adaptive response to overcome the effects of ROS.

Last studies investigated oxidative damage biomarkers after training are controversial. Some of them reported no difference in oxidative damage after training<sup>[37]</sup>. Others reported decrease<sup>[15,27]</sup> and increase<sup>[13]</sup> in oxidative damage biomarkers. Our results showed higher levels of oxidative damage biomarkers in trained groups. This incoherence may be due to either training protocols that have been used or the kind of biomarkers that have been measured.

The effects of intensity and duration of training protocols are studied in many researches<sup>[2,26]</sup>. They found that high intensity and more time are more effective in elevation of oxidative indexes. No study showed the effect of frequency on the body in humans. Here we investigated the effect of low and high training frequency on antioxidant defense at the same intensity and time in non-athlete peoples. Souza-Rabbo et al.<sup>[31]</sup> reported the effect of different training frequency on oxidative stress in liver and heart of rats. He found that antioxidant enzyme activity and lipid damage levels in high frequency trained group was lower than group with low frequency training. It is inconsistent with our results that showed no significant difference in oxidative damage between high and low frequency groups. This incoherence may be due to type of target tissues and the same volume of training between groups. In addition, the volume of exercise in Souza-Rabbo et al.<sup>[31]</sup> research was not the same between trained groups.

Our results showed that increase in antioxidant enzymes activity is more remarkable in trained group with high frequency. It may be due to the short intervals between exercise sessions and more challenge of body against free radicals. In group B, because of more intervals between exercise sessions, body had more time to recovery.

In conclusion, this study suggests that high frequency training increases the antioxidant defense against free radical formed during exercise. Nevertheless, we found higher oxidative stress and damage together with this elevation in antioxidant defense. More studies are necessary to design training protocols that increase antioxidant enzyme activities and decrease the oxidative damage.

## ACKNOWLEDGEMENT

This work was supported in part by a conjoint grant from research Ministry of Tarbiat Moalem University of Tehran.

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