Full Length Research Paper

Effects of endurance training on exhaustive exercise-induced oxidative stress markers

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This study was aimed to investigate the effects of endurance training on lipid peroxidation and antioxidant enzyme levels in young adults. Twenty four male students participated in this study. Subjects were divided into two groups as training group (n=12) and control group (n=12). Subjects in the training groups performed running exercise 25 to 60 min/day, three days/week for eight weeks at an intensity of 50 to 70% of target heart rate. Blood samples collected at rest and after exhaustive exercise before and after 8 weeks, were analyzed for the determination of plasma lipid hydroperoxide (LOOH) level, and activities of glutathione peroxidase (GPx), catalase (CAT) and lactate dehydrogenase (LDH). LOOH level in the training group significantly decreased after the post-training period (p<0.05), but did not alter in the control group (p>0.05). LDH activities significantly increased both control and training groups after the exhaustive exercise (p<0.05). LDH activity was found significantly higher in control group than training group both at rest and after exhaustive exercise (p<0.05). GPx was not significantly altered by training and exhaustive exercise in both groups (p>0.05). In the training group, exhaustive exercise significantly increased CAT level after training period (p<0.05). Consequently, it can be said that endurance training is effective in prevention of lipid peroxidation caused by exhaustive exercise.

Key words: Endurance training, oxidative stress, antioxidant.

INTRODUCTION

Aerobic exercise can increase oxygen consumption by 10 to 20 folds in whole body, and 100 to 200 folds in skeletal muscle (Packer, 1997). Increased oxygen consumption during exercise enhances reactive oxygen species formation (Bailey et al., 2003) and can lead to oxidative stress (Alessio, 1993; Williams et al., 2006). Oxidative stress is defined as an imbalance between oxidants and antioxidants in favor of oxidants, potentially leading to damage (Sies, 2000). Packer (1997) claim that the benefits of regular physical exercise are well known, including improved cardiovascular system function, enhanced energy metabolism and antioxidant defence, greater muscle strength and endurance, and decreased osteoporosis.

However, Ji (1999) suggest that heavy physical exercise enhances free radical production in skeletal muscle and other tissues. Exercise causes an increase in free-radical formation only when it is exhaustive. Changes in indicators of free-radical damage occur only when exercise is exhaustive and are independent of the absolute intensity of exercise (Vina et al., 2000). Several studies provide evidence that regular training decreases oxidative stress and improves antioxidant status (Cakır et al., 2010; Elosua et al., 2003; Radak et al., 1999).

We tested the hypothesis that endurance training would strengthen antioxidant defenses and decrease lipid peroxidation at rest and after acute exhaustive exercise. Hence, in our study, the changes in lipid hydroperoxide as a marker of lipid peroxidation; activities of glutathione peroxidase and catalase as endogenous antioxidant enzymes; and lactate dehydrogenase as a marker of muscle damage, were examined.

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MATERIALS AND METHODS

Study subjects

A total of 24 volunteers (24±3.8 years old) were enrolled in this study. Subjects had not participated in any regular exercise programs within the previous year, but all subjects were moderately active. All participants were nonsmokers, and for at least 2 months before the study, they did not take any vitamins, minerals, or medication that would affect oxidative stress markers. The study was approved by the Ethical Committee of the School of Physical Education and Sports of Selcuk University. All subjects enrolled in this study were university students and gave written, informed consent.

Training and exercise procedures

Participants were randomly divided into two groups: training (T, n=12) and control (C, n=12). The control group was not taken in any programs. Subjects in the training group performed running exercise 25 to 60 min/day, three days/week for eight weeks at an intensity of 50 to 70% of their target heart rate (THR). It was calculated using the Karvonen method (Soucy, 2006), which uses an individual's heart rate reserve in the calculation. While still using the maximum heart rate (MHR) calculation from above (220 age), it incorporates an individual's resting heart rate (RHR) to determine something called their heart rate reserve (HRR), which is equal to MHR-RHR. The theory is that by incorporating RHR, an individual's fitness level is better taking into account. The formula is:

THR= (MHR-RHR)*%intensity + RHR

Body mass index (BMI) was calculated as weight divided by the square of height. To determine the maximum oxygen consumption (VO$_{2max}$), the Bruce Treadmill Protocol was used. One week later participants ran on the treadmill for exhaustive running exercise test; after warming up for 10 min, participants performed exhaustive running exercise test at an intensity corresponding to a speed and slope eliciting 100% VO$_{2max}$ at their highest levels of Bruce Treadmill Protocol. Heart rate was monitored continuously during the test and the recovery period. The criteria for ending exhaustive exercise test were achievement of age adjusted predicted maximum heart rate and subject requests to stop running exercise test. Each subject was tested at the same time of day, to minimize the effects of diurnal biological variation. The same exercise test was repeated 2 days after the last training at the end of 8-week training period. In order to see the effect of the training, the participants were subjected to running exercise until they became exhausted with 100% VO$_{2max}$, at the speed and slope reaching VO$_{2max}$ according to Bruce Test Protocol before starting the training, and blood samples were taken before and after the test.

Biochemical analyses

Blood samples were taken from each of the healthy volunteers after 8 h fast at pre and immediately after the acute exhaustive exercise test. Each subject was tested at the same time of the day (9:00–12:00 AM), to minimize the effects of diurnal biological variation. Blood was drawn from the antecubital vein into an 10 ml Vacutainer tube (EDTA). Plasma was obtained by centrifugation of blood at 2500 rpm for 10 min at +4°C. Obtained plasma was immediately used for biochemical analyses as follows.

Lipid hydroperoxide (LOOH)

LOOH values were determined by using a Lipid Hydroperoxide

Assay Kit (catalog no. 705002; Cayman Chemical Co., Ann Arbor, Michigan, USA) according to the manufacturer's instruction. Hydroperoxides are highly unstable and react readily with ferrous ions to produce ferric ions. The resulting ferric ions were detected using thiocyanate ion as the chromogen. Because this method relies on the measurement of ferric ions generated during the reaction, ferric ions present in the sample are a potential source of error. In addition, many biologic samples contain H$_2$O$_2$, which reacts readily with ferrous ions to give an over estimation of LOOH. These problems are easily circumvented by performing the assay in chloroform, as we did in this study.

Glutathione peroxidase (GPx)

GPx were analysed by using a Glutathione Peroxidase Assay Kit (catalog no. 703102; Cayman Chemical Co., Ann Arbor, Michigan, USA) according to the manufacturer's instruction. This enzyme catalyses the reduction of hydroperoxides, including H$_2$O$_2$, by reduced glutathione and functions to protect the cell from oxidative damage.

Catalase (EC 1.11.1.6; 2H$_2$O$_2$ oxidoreductase)

The catalase (CAT) assay kit was obtained from Cayman Chemical (catalog no. 707002, Cayman Chemical Co., Ann Arbor, Michigan, USA) and the assays were conducted according to their instructions. The kit consists of assay buffer, sample buffer, formaldehyde standard, catalase, potassium hydroxide, methanol, hydrogen peroxide, purpald, and potassium periodate. After reaction of the enzyme with methanol in the presence of H$_2$O$_2$, formaldehyde is produced, and is measured by a spectrophotometric method with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole as the chromogen. Purpald forms a bicyclic heterocycle with aldehydes, and this complex changes from colorless to purple upon oxidation.

Lactate dehydrogenase (LDH)

LDH catalyzes the reduction of pyruvate by reduced nicotinamide adenine dinucleotide. The rate of decrease in the concentration of NADPH, measured photometrically, is proportional to the catalytic concentration of LDH present in the plasma (Ref. No. 1001260; Spinreact S.A. Girona, Spain).

Statistical analysis

Statistical analyses were performed on SPSS version 15.0. A two-way repeated measures analysis of variance was used to analyze changes in physical characteristics from pre-training to post-training. For a significant interaction effect, within subject factors were analyzed using the Bonferroni corrected paired samples t-test. Unpaired t-tests were used to compare mean values between groups at both the beginning and end of the training period. Statistical significance was set at a $p < 0.05$ level and data are expressed as mean ± SEM.

RESULTS

The physical characteristics of the subjects are presented in Table 1. Body composition changes before and after the training tested by two-way repeated measures analysis were given in Table 1. While significant

Table 1. Changes in physical characteristics during the experimental period.

	Group	Mean ± SEM		Time	Time x Group	Group
		Pre-training	Post-training			
Body weight (kg)	Control	71.1±1.8	70.8±1.9	4.63*	1.61	0.34
	Training	73.1±2.1	72.1±2.0[#]			
BMI (kg/m^2)	Control	23.5±0.6	23.4±0.7	4.48*	1.35	0.04
	Training	23.4±0.6	23.1±0.6 [#]			
Body fat (%)	Control	12.2±0.3	12.0±0.4	33.85	10.63	0.04
	Training	12.7±0.7	11.7±0.7[#]			
VO$_{2max}$ (ml/kg/min)	Control	51.5±1.2	52.1±1.1	41.26	23.78	2.14
	Training	51.8±1.3	56.5±0.9[#†]			

*$p < 0.05$, compared between training and control groups (two-way repeated measures ANOVA). [#] $p < 0.05$, significantly different from pre-training levels.[†] $p < 0.05$, significantly different from the control group at post-training. Training group n=12, control group n=12.

difference was determined in time factor at body weight (F=4.63) and BMI (F=4.48) variance values of the subjects in training and control group (p<0.05), no difference was found between group-time and group factors. While significant difference was determined between time (F=33.85) and group-time interaction (F=10.63) in body fat values (p<0.05), no difference was found in group factor. In the training group, body weight, BMI and body fat values decreased significantly after the training. There was no significant change in the control group. While significant difference was determined in time (F=41.26) and group-time (F=23.78) factors in VO$_{2max}$ variance values of the subjects (p<0.05), no difference was found in group factor. While VO$_{2max}$ increased significantly in training group (p<0.05), it did not change in the control group (Table 2).

No significant time, group, or interaction effects were observed for LOOH at rest and after exhaustive exercise in the pre-training. There was a significant interaction effect (F=6.04) for LOOH levels in post-training but no significant time and group effect. LOOH levels in the training group significantly decreased after the post-training period. There was a significant time effect (F=26.31) for LDH activities in pre-training but no significant interaction and group effect. LDH activities significantly increased both control and training groups after the exhaustive exercise. After the training, there was a significant time (F=29.71) and group (F=12.51) effects for LDH activities in post-training but no significant interaction effect. Also, LDH activities in the control group were higher than the training group at rest and after exhaustive exercise (p<0.05). No significant time, group, or interaction effects were observed for GPx at rest and after exhaustive exercise in the pre- and post-training. There was a significant interaction effect for CAT levels in pre- (F=6.24) and post-training (F=3.37) but no significant

time and group effect. Exhaustive exercise increased CAT levels in the training group increased after the post-training period. (p<0.05).

DISCUSSION

The aim of this study was to investigate the effects of endurance training on exhaustive exercise-induced lipid peroxidation and antioxidant enzyme levels. The major findings of this study are that LOOH level in the training group significantly decreased after the post-training period, but did not alter in the control group. LDH activities were found significantly higher in control group than the training group both at rest and after exhaustive exercise. GPx was not significantly affected by training and exhaustive exercise in both groups. In the training group, exhaustive exercise significantly increased CAT level after the training period.

At present, it appears that all forms of exercise, both aerobic and anaerobic, possess the potential to result in increased reactive oxygen/nitrogen species production and subsequent oxidative stress (Fisher-Wellman and Bloomer, 2009). In our study, exhaustive exercise did not significantly change LOOH values in neither training nor control group before training period of exhaustive exercise. However, at the end of 8 week exercise program, it significantly decreased LOOH values after exhaustive exercise. Nevertheless, no agreement was reached about whether lipid peroxidation was affected by exercise or not. Fogarty et al. (2011) determined that LOOH level, which is an indicator of lipid peroxidation, increased during 3x5 min exercise with 40, 70 and 100% VO$_{2max}$. Similarly, Ajmani et al. (2003) stated that maximal running exercise increased LOOH level significantly. On the other hand, González et al. (2008) reported that the increase in uric acid

Table 2. Changes in LOOH, LDH, GPx and CAT levels in the control and training groups before and after exhaustive exercise in the pre- and post-training periods.

		Group	Rest Mean ± SEM	Exhausted Mean ± SEM	Time	Time x Group	Group
LOOH (nmol)	Pre-training	Control	5.1±0.1	5.2±0.1	0.14	0.04	0.37
		Training	5.0±0.2	5.1±0.1			
		Control	4.8±0.2	5.0±0.1		*	
	Post-training	Training	5.3±0.1†	4.6±0.2#	1.74	6.04	0.12
LDH (U/L)	Pre-training	Control	261.8±17.3	342.0±27.9#	26.31 *	0.51	2.59
		Training	287.5±16.0	393.6±21.8			
		Control	218.8±12.0	290.5±19.4#	*		
	Post-training	Training	181.2±8.9†	217.0±9.3#†	29.71	3.32	12.51
GPx (nmol/min/ml)	Pre-training	Control	32.4±3.2	28.5±2.0	1.27	0.31	0.22
		Training	32.3±2.8	31.0±1.2			
	Post-training	Control	45.0±7.1	42.5±4.7	0.16	0.62	3.19
		Training	50.6±5.2	58.1±7.2			
CAT (nmol/min/ml)	Pre-training	Control	26.2±2.4	36.8±3.4#†	1.92	6.24 *	3.31
		Training	27.9±2.6	24.9±2.6			
		Control	21.9±2.2	20.9±2.7		*	
	Post-training	Training	13.4±1.7†	20.6±1.7#	2.45	3.37	3.82

*$p < 0.05$, compared between training and control groups (two-way repeated measures ANOVA). # $p < 0.05$, significantly different from pre-training levels. † $p < 0.05$, significantly different from the control group at post-training. Training group n=12, control group n=12.

and total antioxidant activity repressed LOOH level after aerobic running exercise.

Exercise has not been conclusively shown to induce oxidative stress or oxidative damage in all modes. It is likely that factors such as duration, intensity, fitness, breed, athletic ability, health, and environmental conditions all have an impact on the occurrence or severity of oxidative stress and damage (Deaton and Marlin, 2003). LDH which is the most useful serum markers of muscle injury is an enzyme protein that interconverts pyruvate and lactate, with concomitant interconversion of NADH and NAD (Brancaccio et al., 2010). In our study, while exhaustive exercise significantly increased LDH activities in both groups, LDH activity was found higher in control group than the training group both at rest and after exhaustive exercise. Similarly, Burneiko et al. (2006) stated that aerobic exercises continuing for 8 weeks decreased LDH activities. In contrast to these findings, it was reported that sportsmen had higher LDH values than sedentary individuals in the studies performed (Brites et al., 2006; Evelson et al., 2002).

Cells continuously produce free radicals and reactive oxygen species (ROS) as part of their metabolic processes but free radicals are neutralized by antioxidant defense system (Matsuo and Kaneko, 2000). CAT and GPx provide the primary defense against ROS generated during exercise, and activities of these enzymes are known to increase in response to exercise in both animal and human studies (Ji, 1999). In our study, GPx was not significantly affected by training and exhaustive exercise in both groups. However, exhaustive exercise significantly increased CAT level after training period in the training group. While many researches did not determine any change in GPx enzyme activity with acute maximal exercise after endurance training period (Miyazaki et al., 2001; Özta an et al., 2004), some determined an increase (Elosua et al., 2003; Fatouros et al., 2004; Servais et al., 2003), and some determined a decrease (Balakrishnan and Anuradha, 1998; Gül et al., 2006). Similarly again, while some researchers determined no change in CAT enzyme activity (Balakrishnan and Anuradha, 1998; Gül et al., 2006; Miyazaki et al., 2001), there are also other researches who have found an increase (Alessio and Goldfarb, 1988; Poprzecki et al., 1997; Servais et al., 2003).

Among possible reasons for the contradictions in studies towards determining exercise-related lipid peroxidation and antioxidant status; the differences between type, duration and intensity of the exercise applied, type of the subjects and their qualities, the periods during which measurements are made before and after exercise and the methods used and doing the research in different configurations and environments may be shown. This study has some limitations. One of them is that the

participants of training program were mid-level active individuals. And another one is that a limited number of oxidative stress markers were examined in the study. Additionally, the participants were informed about dietary measures but not controlled.

Conclusion

In conclusion, it can be said that exhaustive exercise increases LDH activities which are the indicator of muscle damage, and that the regular endurance training is effective in prevention of lipid peroxidation caused by exhaustive exercise.

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