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Aerobic and anaerobic training effects on the antioxidant enzymes of the blood

Semih **Selamoglu**[1], Faruk **Turgay**[1], Berkant Muammer **Kayatekin**[2], Sevil **Gönenc**[2], Çetin **Yslegen**[3]

[1] Izmir Sports Health Center
[2] Department of Physiology, Dokuz Eylül University Medical School
[3] Department of Sports Medicine, Ege University Medical School, Izmir, Turkey

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The purpose of the present study was to investigate the effects of aerobic and anaerobic training on serum lipid peroxidation levels and on antioxidant enzyme activities.

Long distance runners for aerobic training group, and wrestlers for anaerobic training group were chosen. Non-sporting men were used as control group. When the aerobic power was compared; indirect VO_2max of long-distance runners were found higher than wrestlers and control group ($p<0.001$, $p<0.001$). When lipid peroxidation levels were compared; levels of the thiobarbituric acid reactive substances (TBARS) of long distance runners were found to be lower than those in the control group ($p<0.05$), but similar to those found in wrestlers. Comparison of antioxidant enzyme activities in erythrocytes show that there were no significant difference among the groups in superoxide dismutase enzyme activities, but glutathione peroxidase (GPx) activity of long distance runners was higher than that measured in wrestlers ($p<0.05$).

These results suggest that aerobic training increased in erythrocytes GPx activity with a subsequent decrease in plasma TBARS levels but anaerobic training had no effect on this process.

Keywords: superoxide dismutase, glutathione peroxidase, thiobarbituric acid, reactive substances, exercise, aerobic and anaerobic training, non sporting men, long-distance runners, antioxidant enzyme activities

Increased energy demand during physical exercise, especially of the aerobic type, necessitates a multifold increase in oxygen supply to active tissues. During exercise,

Correspondence should be addressed to
Berkant Muammer Kayatekin, MD
Department of Physiology,
Dokuz Eylül University Medical School
35340 Balçova, Izmir, Turkey
Fax: 00-90-232-2590541
E-mail: kayabm@deu.edu.tr

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bodily oxygen consumption is greatly increased, up to 10- to 15-fold greater than resting levels [9]. Oxygen-centred radicals are produced in the intermediate metabolism [4]. Most of the oxygen consumed in the mitochondria is utilized to produce adenosine 5'-triphosphate, but during oxidative phosphorylation the superoxide radicals, hydrogen peroxide, and hydroxyl radicals are produced by the univalent reduction of oxygen and leak out of the electron transfer chain [6]. As a whole, they are classified as reactive oxygen species (ROS) and are responsible for a series of biochemical and physiological changes, namely oxidative stress. The ROS released cause the lipid peroxidation of polyunsaturated fatty acids in the biological membranes and blood, inducing alterations of the cell functions [7]. Lipid peroxides readily decompose to liberate highly reactive carbonyle fragments such as malondialdehyde. Malondialdehyde (MDA) was the major species responsible for thiobarbituric acid reactive substances (TBARS) [8].

Strenuous physical exercise induces oxidative damage to lipids in various tissues [22, 23]. In resting state the body is equipped with both non-enzymatic and enzymatic antioxidant reserves to prevent the potentially harmful effects of ROS [13]. The fine physiological balance between oxidative reactions and antioxidant capacity may be perturbed by intense physical activity. Antioxidant defence systems preserve homeostasis for normal cell function at rest and perhaps during mild-oxidative stress. Primary components of the physiological antioxidant defence are superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). SOD catalyzes the dismutation of superoxide to O_2 and H_2O_2, which catalase (CAT) converts to water and O_2. GPx can reduce H_2O_2 to form glutathione disulphide and water [5].

Large number of studies have tested the effect of a variety of endurance exercise training regimens on antioxidant defences, but information on the effect of anaerobic training on antioxidant defences is scanty. The aim of the present study was to assess the effects of aerobic and anaerobic training on serum lipid peroxidation levels and on antioxidant enzymatic activities in erythrocytes.

Materials and Methods

This study consisted of 33 non-smoking males (17.61±2.28 years, BMI 21.94± 0.63); including 11 long-distance runners (aerobic training group) and 11 wrestlers (anaerobic training group) who have been doing sport for average 5.18±0.70 years on the awerage and in control group. The control subjects did not perform any regular physical activity before the study. All the experiments were performed in according to the Helsinki Declaration.

Subjects attended the laboratory in the morning, after a 12 h fast and a 10 ml blood sample was obtained from an antecubital vein. Blood samples were collected 48

hours after the termination of the training in order to minimise the residual effect of the last exercise. Heparinized venous whole blood was used for measuring erythrocyte antioxidant enzymes (SOD and GPx) activities. Blood hemoglobin values for calculating enzyme activities were determined by using Coulter Counter. Plasma TBARS levels were measured as an indicator of lipid peroxidation.

Determination of SOD activity

Erythrocyte SOD was determined with a Randox test combination (Randox, Crumlin, UK). Xanthine and xanthine oxidase were used to generate superoxide radicals reacting with 2-(4-iodophenyl) 3-(4-nitrophenol)-5 phenyl tetrazolium chloride (INT) to form a red formazan dye. The concentration of the substrates were 0.075 μmol for xanthine and 0.037 mmol for INT. Superoxide dismutase inhibits this reaction by converting the superoxide radical to oxygen. A SOD unit inhibits the rate of reduction of INT by 50% in a complex system with xanthine and xanthine oxidase. Because of the small linearity range of the test, the sample was diluted so that the percentage of inhibition fell between 30% and 60%. A standard curve was prepared, using the kit standard, and the value for the diluted sample was read from this curve. SOD activity was measured at 505 nm on a Shimadzu UV-1201v spectrometer on hemolysates of washed erythrocytes obtained by centrifugation of whole blood. Results were expressed in SOD U/g hemoglobin.

Determination of GPx activity

GPx was also determined with a Randox test combination (Randox, Crumlin, UK). GPx catalyses the oxidation of glutathione (at a concentration of 5 mmol) by cumene hydroperoxide according to the method of Paglia and Valentine [21]. In the presence of glutathione reductase (at a concentration >0.75×10-3 U) and 0.35 mmol NADPH, the oxidised glutathione was immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm was measured at 37 °C. The assay was performed on a hemolysate of washed erythrocytes obtained from the mixing of 0.05 ml whole blood with 1 ml cold diluting agent and 1 ml Drabkin reagent. The GPx unit was defined as the enzyme activity necessary to convert 1 mmol of NADPH to NADP in 1 minute. The activity of GPx is expressed in U/g hemoglobin.

Determination of TBARS level

In a modified Yagi method [24], 0.05 ml of blood was sampled with a pipette for determination of blood cells and placed in 1.0 ml of normal saline in a centrifuge tube. After gently shaking, the tube was spun at 3000 rpm for 10 minutes and 0.5 ml of the supernatant was transferred to another centrifuge tube. The 4.0 ml of 1/12 N H_2SO_4 was added to this solution and the mixture was shaken gently. Then 0.5 ml of 10% phosphotungstic acid was added and mixed. After standing at room temperature for 5 minutes, the mixture was centrifuged at 3000 rpm for 10 min. After the supernatant was discarded, the sediment was mixed with 2.0 ml of N/12 H_2SO_4 and 0.3 ml of 10% phosphotungstic acid and the mixture was centrifuged. The sediment was suspended in 4.0 ml of distilled water and 1.0 ml of thiobarbituric acid (TBA) reagent was added. The reaction mixture was heated for 60 min at 100 °C in a water bath. After cooling with tap water, 5.0 ml of n-butanol was added and the mixture was shaken vigorously, then centrifuged at 3000 rpm for 15 min. Finally, the n-butanol layer was taken for spectophotometric measurement at 532 nm. A standard curve was prepared using the MDA standard (1, 1, 3, 3-tetraethoxypropane) and the value for the plasma was read from this curve. The results were expressed as nmol/ml.

Determination of VO_2max

VO_2max was determined indirectly by Astrand's method using Monarck bicycle ergometer [3].

Statistical analysis

All results were expressed as mean ±S.E.M. The statistical analysis of the data was performed using Mann–Whitney U test. The significance was set at $p<0.05$.

Results

When the effect of exercise on aerobic power was examined; indirect VO_2max of long-distance runners were found higher than wrestlers and non-sporting men (respectively, $p<0.001$, $p<0.001$, Table I).

Table I

Indirect VO_2max values, SOD, GPx activities and TBARS levels of sportsmen and of the control group

Groups	VO_2max (ml/kg/min)	SOD (U/gHb)	GPx (U/gHb)	TBARS (nmol/ml)
Control	47.40 ± 1.57	1150.36 ± 51.60	42.96 ± 4.86	1.43 ± 0.09
Distance runners	62.30 ± 1.85***	1059.27 ± 111.29	56.62 ± 3.68**	1.08 ± 0.10*
Wrestlers	49.71 ± 1.49	1269.61 ± 66.44	45.54 ± 3.13	1.15 ± 0.15

Results were presented as means ±S.E.M.

* Lower than control $p<0.05$

** Higher than wrestlers $p<0.05$

*** Higher than wrestlers and control $p<0.001$

When plasma lipid peroxidation levels and antioxidant enzymatic activities were investigated; TBARS levels of long-distance runners were lower than those of non-sporting men ($p<0.05$) but similar to those measured in wrestlers. GPx enzyme activity of long-distance runners was higher than those of wrestlers ($p<0.05$) but similar to those found in the non-sporting males. There were no significant difference among the groups in SOD enzyme activities (Table I).

A positive correlation between the subject's VO_2max and GPx activity was found ($r=0.53$, $p<0.01$). A negative correlation between the subject's VO_2max and plasma MDA level was found ($r=–0.38$, $p<0.05$).

Discussion

Increased oxygen utilization during exercise cause generation of free radicals [11]. Chronic aerobic training has been claimed to reduce exercise-increased lipoperoxidation by improving the body's defence capabilities against free radicals generation, likely as a result of an adaptive increase in the activities of the scavenger enzyme systems [2]. Scientific literature on the effect of anaerobic training on antioxidant defence system and on lipid peroxidation is scanty [17].

Human erythrocytes are well equipped with the enzymes SOD, catalase and GPx, that protect the cells against the accumulation of superoxide radical and/or hydrogen peroxide normally produced during the oxidation of hemoglobin [19]. It has been reported that endurance training elevates the antioxidant enzyme activities in blood at rest and during post-exercise recovery [14, 17]. However, a controversy still exists as to which enzyme and under what condition an enzyme can be activated [11, 12, 15, 16]. Available data suggest that each of the antioxidant systems may have a different

response to acute and chronic exercise depending upon their biochemical and molecular mechanism of regulation [10]. Mena et al. described that under resting conditions the SOD and GPx activities were higher in cyclists than in the control group [18]. Marzatico et al. determined that blood SOD and GPx activities were higher in marathon runners and sprinters than in controls [17]. Ohno et al. have reported that in sedentary students after a brief physical exercise no increase in erythrocyte SOD activity was found [19]. In another study, resting blood SOD and GPx activities were no different in jump-trained (volleyball players) compared with untrained subjects [20]. It has been reported that blood GPx activity increased after swim training program in mice [14].

The results from the present investigation demonstrated that SOD activity was unaffected by aerobic and anaerobic training. GPx enzyme activities of long-distance runners were higher than those detected in wrestlers. Despite exercise-induced free radical changes, there is a positive side to oxidative stress associated with regular exercise [1]. Subjects with high aerobic power show significantly greater antioxidant enzyme activity [17, 23]. It has been reported that there exist a good correlation between exercise endurance time and GPx activity [1]. In this study, a positive correlation between the subject's VO_2max and GPx activity was found. This could further show how even aerobic training is able to prevent the toxic effects of lipid peroxidation.

MDA is the end product of lipid peroxidation and is a well-known parameter for determining the increased free radical formation in the body. It has been reported that lipid peroxidation levels are lower in endurance-trained than in untrained animals [2]. The knowledge on the effect of training on lipid peroxidation in humans is sparse equivocal. Ohno et al. [19] and Jenkins et al. [12] determined that plasma MDA levels were decreased related to training adaptation. However, Marzatico et al. found that resting plasma MDA levels of marathon runners and sprinters are higher than control levels [17]. Results of studies on lipid peroxidation induced by exercise are actually inconsistent due to the wide variety of methods employed and the differences in exercise protocols (e.g. type, duration and intensity of exercise). It was observed that resting plasma MDA negative by correlated with the aerobic capacity of the individuals, suggesting a protective effect of physical fitness [23].

In the present study, when resting plasma lipid peroxidation levels were examined; a negative correlation between the subject's VO_2max and plasma TBARS level was found. TBARS levels of long distance runners were lower than control levels but TBARS levels of wrestlers did not differ from those found in controls.

In conclusion, these results suggest that aerobic training increased GPx activity in erythrocytes with a subsequent decrease in plasma TBARS levels while anaerobic training had no effect on this process.

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