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Effect of Exercise on Blood Antioxidant Status and Erythrocyte Lipid Peroxidation: Role of Dietary Supplementation of Vitamin E

Abstract: We tested the effects of moderate physical exercise on the blood antioxidant capacity and erythrocyte lipid peroxidation in 21 Wistar albino rats. Erythrocyte superoxide dismutase (SOD) activity increased significantly ($p<0.05$) in control exercised animals (C-Ex), but catalase activity did not change. SOD activity was decreased by dietary supplementation of vitamin E ($p<0.05$). In vitamin E supplemented group (E-Ex), catalase activity was reduced in comparision to C-Ex group. Total glutathione (total GSH) level was unaffected by the exercise. However, significant reduction was observed in reduced glutathione (GSH), whereas oxidized glutathione (GSSG) increased ($p<0.01$ and $p<0.001$, respectively). In E-Ex animals, total GSH and GSH were increased in comparison to C-Ex group. GSH/GSSG ratio decreased abnormally in both exercised groups ($p<0.001$). Serum cholesterol and uric acid levels increased significantly after exercise ($p<0.05$). The susceptibility of eryhrocytes to in vitro peroxidation increased in C-Ex and E-Ex animals ($p<0.001$ and $p<0.01$, respectively). Elevated malondialdehyde (MDA) concentrtaion in serum attained statistical significance after exercise. However, this elevation was prevented by vitamin E supplementation.

Our results indicated that moderate intensive treadmill running exercise was sufficient to result in muscle damage and increases in the susceptibility of erythrocytes to in vitro peroxidation. In addition, dietary supplementation of vitamin E is able to minimize oxidative damage caused by exercise.

Key Words: Moderate exercise, lipid peroxidation, erythrocyte, Vitamin E.

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Introduction

Experimental studies have shown that strenuous exercise promotes free radical formation and lipid peroxidation in skeletal muscle and erythrocytes. The rise in free radical concentrations could exceed the protective capacity of cell antioxidant defense systems (1, 2). Exhaustive exercise in rats leads to the depletion of glutathione (GSH) levels, and the training programme induced through treadmill running was demonstrated to increase oxidative capacity in liver, muscle and blood (3, 4). Lew et al, reported that rats exercised exhaustion by running show large increases in the total glutathione and glutathione disulphide in plasma after exercise. The ratio of reduced GSH to oxidized glutathione decreases in the plasma, liver, and skeletal muscle of exhausted rats (5).

An increase oxidative stress is also supported by studies showing an induction of antioxidant activity that occurs during exercise (6, 7, 8, 9), as well as a training response, where animals that were exercised regularly demonstrated lower malondialdehyde (MDA) in active tissue when compared with sedentary controls (3). Furthermore, in another study, enhanced erythrocyte susceptibility to peroxidation after exercise has been reported by Duthie et al, (2). However, it has been shown that during high intensity rowing training caused no apparent oxidant stress and muscle damage (10).

Most of the previous works on exercise and oxidative stress have used high intensity exercise regimes, including exhaustive exercise (8, 15, 16). The effects of moderate intensity exercise, which is more normally experienced, is not well known. Accordingly, the purpose of this study was to investigate the susceptibility to in vitro peroxidation of erythrocytes immediately after moderate exercise. In addition, we examined whether treadmill running training would affect selected markers of oxidant stress and dietary suplementation of vitamin E would

	CONTROL (n=7)	C-Ex (n=7)	E-Ex (n=7)
SOD (IU.gHb^{-1})	2402.61±195.77	2638.90±120.88*	2410.61±150.48♦
Catalase (k.gHb^{-1})	1936±255	1969±246	1617±186♦
Total GSH (mg.gHb^{-1})	0.524±0.01	0.495±0.04	0.560±0.02**♦♦
Reduced GSH (mg.gHb^{-1})	0.515±0.03	0.446±0.03**	0.531±0.04♦♦
GSSG (mg.gHb^{-1})	0.009±0.002	0.047±0.008***	0.024±0.011*♦♦
GSH/GSSG	59.53±14.91	9.69±1.86***	26.11±11.00***♦♦♦

Table 1. Enzymatic and non-enzymatic antioxidant defense system in erythrocytes.

Values are expressed mean±SD

Significant differences from control values, *P<0.05, **p<0.01, ***p<0.001.

Significat differences from C-Ex values ♦p<0.05, ♦♦p<0.01, ♦♦♦p<0.001

	CONTROL (n=7)	C-Ex (n=7)	E-Ex (n=7)
Serum vit. E (µg.ml^{-1})	5.35±0.03	2.40±0.24***	3.38±0.25*** ♦♦♦
Uric acid (mg.dl^{-1})	3.77±0.83	4.80±0.55*	4.29±0.56
Cholesterol (mg.dl^{-1})	58.14±4.22	64.86±6.20*	62.71±6.95

Table 2. Non-enzymatic antioxidant, cholesterol in serum.

Values are mean ± SD

Statistical differences from control values *p<0.05, ***p<0.001

Statistical differences from C-Ex values ♦♦♦ p<0.001

protect erythrocytes from possible oxidative stress in exercise.

Materials and Methods

Animals and exercise programme: Wistar strain albino rats of three-month old group maintained one per cage in a thermostatic (22±3℃) 12 hr light/dark cycles were fed either a control diet or a vitamin E supplemented diet. Vit E was dissolved in ethyl alchol (99.9%) and supplemented to the control diet daily in the form of D-α-tocopherol (Sigma Chemical Company St Louis, MO). The final concentration of vitamin E in the control diet was 80.5 IU.kg^{-1} diet and that of the supplemented diet was 220 IU.kg^{-1} diet. Twenty-one

male rats were divided into three groups: Control (C, n=7), Exercise (C-Ex, n=7) and Vit.E+Exercise (E-Ex, n=7). Exercise programme consisted of level treadmill running at a velocity of 900 m.hr^{-1} for 30 min. day^{-1} regularly every day for four weeks. The exercised and control animals were killed at the same time immediately after the exercise, and blood was isolated and taken for analysis.

Measurements: Activity of superoxide dismutase (SOD) was measured by the method of winterbourn et al., which is based on the inhibition of the reduction of nitroblue tetrazolium by O_2 produced via photoreduction of riboflavin (17). Fifty percent inhibition was defined as 1 Unit of SOD activity. Catalase activity was determined according to the method of Aebi (18). Published methods

	CONTROL (n=7)	C-Ex (n=7)	E-Ex (n=7)
CK (IU.L^{-1})	288.71±45.23	578.29±64.50***	416.14±43.67***♦♦♦
LDH (IU.L^{-1})	238.29±14.42	429.57±64.27***	269.43±38.54♦♦♦
Serum MDA (nmol.ml^{-1})	2.58±0.30	3.47±0.28***	2.40±0.14♦♦

Table 3. Nonspecific indices of muscle damage and lipid peroxidation in serum.

Values are mean ± SD
*Statistical differences from control values **p<0.01, ***p<0.001*
Statistical differences from C-Ex values ♦♦p<0.01, ♦♦♦p<0.001

	CONTROL (n=7)	C-Ex (n=7)	E-Ex (n=7)
α-tocopherol (μg.gHb^{-1})	14.66±1.59	13.03±1.07*	15.87±1.17*♦♦
MDA (μmol.gHb^{-1})	0.64±0.10	1.46±0.16***	0.91±0.18**♦♦

Table 4. Effects of exercise and vit. E Supplementation on hydrogen peroxide induced erythrocyte susceptibility to peroxidation and on erythrocyte α-tocopherol concentrations.

Values are mean ± SD
*Statistical differences from control values *p<0.05, **p<0.01, ***p<0.001*
Statistical differences from C-Ex values ♦♦p<0.01

were used for the concentration of reduced and oxidized Glutathione (19). Vitamin E levels in the serum, erythrocytes and diet were analyzed by the modified method of Seits et al., (20) using D-α-tocopherol as an internal standard. Serum, erythrocytes and diet samples, after initial exraction with n-hexane, were injected on to the Nucleosil 5C 18 column and separated on Jasco JCL-6000 HPLC. The solvent used was methanol (HPLC grade-SIGMA) and the eluant was monitored on fluorescence detector (Ex 295 nm, Em 340 nm). Lipid peroxide concentration was measured as the total thiobarbituric acid reactive substances (TBARS) as described by Asakawa and Matsushita (21). The results are expressed as malondialdehyde (MDA) equivalents or as TBARS. The susceptibility of erythrocytes to in vitro peroxidation was measured by the method of Duthie et al. (2).

Plasma total cholesterol, creatine kinase, lactatdehydrogenase and uric acid concentrations were measured by Abbott Spectrum autoanalyzer. Erythrocytes osmotic fragility was determined by the method of Guest (22).

Statistical Analysis: Student's 't' test was used to analyze the differences between control and exercised groups. The data are given as mean±SD.

Results

SOD, Catalase activity and glutathione: SOD and catalase activities in erythrocytes in response to exercise are reported in Table 1. As compared to sedentary control, SOD activity increased significantly (p<0.05), but catalase activity did not change in C-Ex animals. Activities of these enzymes had a tendency to decrease in E-Ex animals. In total GSH level, no significant difference was observed between sedentary control and C-Ex group. However, significant reduction was determined in GSH, whereas GSSG increased in C-Ex animals (p<0.01, p<0.001, respectively). Suplementation of vitamin E affected positively the total GSH, GSH and GSSG levels compared to C-Ex group. The decrease in GSH/GSSG ratio in exercise group is mainly due to an increase of GSSG in exercise (p<0.001).

Serum Vit. E, Uric acide and cholesterol: Decreased serum vitamin E levels in C-Ex and E-Ex animals, were statistically significant (p<0.001). Exercise induced serum vitamin E depletion was partly prevented by vitamin E
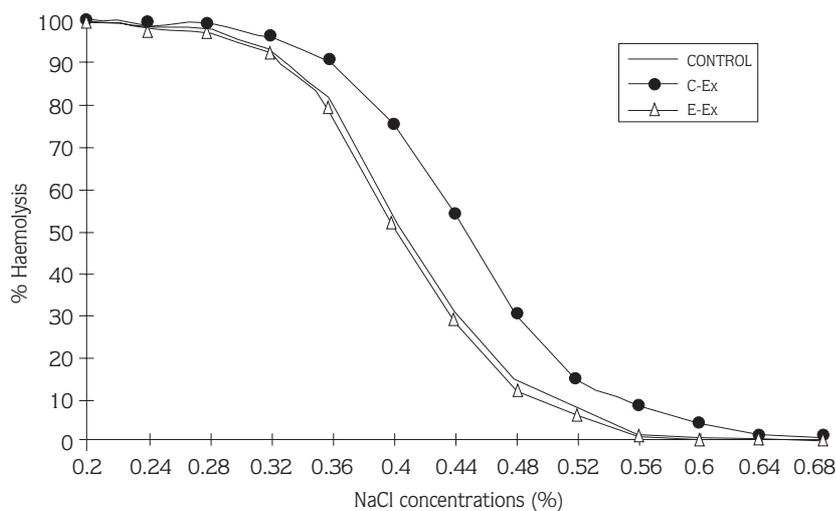
supplementation. In addition, there was a significant increase in serum cholesterol and uric acid levels in C-Ex group (p<0.05, Table 2).

Plasma CK and LDH activities and serum MDA level: As shown in Table 3, plasma CK and LDH activites increased significantly after exercise (p<0.001). These changes were reflected by increases in the MDA as an index of lipid peroxidation. The susceptibility of erythrocytes to in vitro peroxidation increased in C-Ex and E-Ex animals (p<0.001, p<0.01, respectively, Table 4). Osmotic fragility of erythrocytes increased after exercise in C-Ex group, but there was similarity in osmotic fragility of erythrocytes between vitamin E supplemented and control group animals (Figure 1).

Discussion

The rise in oxygen utilization during physical exercise may lead to increase in metabolic leakage of damaging free radicals of oxygen from the mitochondria into the cytosol, resulting in the formation of lipid peroxide (1, 23, 24, 25). The MDA production has been considered as basic reaction in the membrane alterations due to free radical interaction with polyunsaturated fatty acids (3, 15). Elevations in serum levels of the enzymes CK and LDH following various forms of physical exercise are well documented (26, 27, 28, 29, 30). Our findings of elevated MDA production, CK and LDH activities after moderate intensive exercise are similar to those reported by Kanter et al, (31), and Apple et al, (26), and suggest that moderate exercise on treadmill running may induce muscle damage. However, in E-Ex group, these parameters were much lower than those in control animals.

It is well known that SOD, Catalase, GSH and Vitamin E play a vital role in the antioxidant defense mechanism of animal cells and tissues. In our study, SOD activity in erythrocytes inrceased in response to exercise in control animals. Since SOD is known to be an enzyme induced by its superoxide radical substrate, its increase in C-EX animal erythrocytes indicates an increased production of the superoxide radical. The increase also might be involved in the elevated generation of OH•. This increase in SOD activity in C-Ex animals also could be responsible for the observed increase in lipid peroxidation. Regularly performed exercise might induce an adaptive enhancement in skeletal muscle and erythrocytes of the defense mechanisms that protect them against free radical damage (6, 32). We found no increase in erythrocytes catalase activity in C-Ex animals. This finding was similar to those of Higuchi et al, (6) and Duthie et al, (2), but was different from the results of Vani et al, (9). Catalase activity decreased in vitamin E suplemented animals after exercise. The decrease in SOD and catalase activities in E-Ex animals in comparison to control animals explains the protection offered by vitamin E.

Previously, large increases have been shown in the total glutathione and oxidized glutathione in plasma after exhaustive running exercise in rats. The ratio of reduced GSH to GSSG decreases in plasma, liver and skeletal muscle of the exhaustive rats (5). Subsequent studies also reported that levels of total glutathione fell in exhaustive exercised rats (3, 4, 33). Also, we found that total GSH and GSSG increased despite low GSH/GSSG ratio in E-Ex animals but, reduced GSH unchanged. Erythrocyte total glutathione did not change significantly in control exercised group. In addition, the decline in reduced glutathione was accompanied by a corresponding increase

in GSSG. Vitamin E supplementation increased total and reduced glutathione from C-Ex levels in spite of exercise. As a result of protective effect of vitamin E, GSSG level decreased in comparison with C-Ex values. Healthy non-stressed cells maintain notably a high intracellular GSH/GSSG ratio to ensure the availability of reduced GSH, and thereby promote active reduction of hydroperoxides through the glutathione redox cycle (34). In the present study, ratio of reduced GSH to GSSG decreased significantly in both exercised groups. The decrease in this ratio mihgt stems from increase in GSSG level.

Serum cholesterol and uric acid levels increased significantly after exercise. These findings indicate that elevated plasma cholesterol and uric acid protect erythrocytes against peroxidant stress, in accordance with the observations of Bereza et al, (35), and Duthie et al, (2).

Lipid peroxidation following exposure to H_2O_2 is abnormally high in the erythrocytes from patients with low plasma vitamin E concentrations (36). The susceptibility of erythrocyte to in vitro peroxidation increased immediately after exercise in both exercised groups, suggesting that moderate intensive treadmill running for 30 days resulted in a reduction in the ability of the erythrocyte to resist oxidation. However, the susceptibility of erythrocyte to in vitro peroxidation in C-Ex animals was significatly higher than that of E-Ex animals. This was not due to a decrease in activities of the antioxidant enzymes determined; on the contrary, it might be related to increase in GSSG and decrease in reduced GSH after exercise. Osmotic fragility of erythrocytes increased after exercise in C-Ex animals, but supplementation of vitamin E maintained osmotic fragility of erythrocytes in control levels.

The results show that moderate intensive exercise, probably by resulting oxidant stress, alters blood antioxidant status, increases susceptibility to peroxidation and osmotic fragility by attenuating antioxidant capacity of erythrocytes. Dietary supplementation of vitamin E in the amounts used in this study serves to lower marker of lipid peroxidation in exercise. Thus, the susceptibility of erythrocytes to peroxidation is decreased, and osmotic fragilitiy of erythrocytes is improved.

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