Tr. J. of Medical Sciences
28 (1998) 643-647

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Changes in blood antioxidant status and lipid peroxidation following distance running

Received: June 18, 1996

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Abstract: To examine the effects of increased O_2 utilization on blood antioxidant status and lipid peroxidation, ten trained male athletes took part in a 20 km distance race. Venous blood samples were removed before and immediately after completion of the half-marathon. Plasma creatine kinase (CK) activity, an index of muscle damage, increased ($p<0.05$) after the race but this was not accompanied by changes in thiobarbutiric acid reactive substance (TBARS), which is an index of lipid peroxidation. Plasma cholesterol and uric acid concentrations significantly increased ($p<0.01$ and $p<0.05$ respectively) but plasma α-topocherol, triglyceride, and the total protein levels did not change ($p>0.05$) after the race. The erythrocyte and leucocyte counts, hematocrit, and hemoglobin levels rose after the race ($p<0.05$, $p<0.001$, $p<0.05$, and $p<0.01$, respectively). However, the mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) did not change. Erythrocyte superoxide dismutase (SOD) and catalase activities were unaffected by the exercise but total glutathione (GSH) and reduced GSH decreased ($p<0.05$) after the race. In the post-race samples, a rise in oxidized glutathione (GSSG) was not statistically significant ($p>0.05$).

The result indicate that, when trained athletes run a comparatively short distance sufficient to cause some degree of muscle damage but insufficient to cause increases in the plasma indices of lipid peroxidation, changes in erythrocyte antioxidant status do occur.

Key Words: Exercise, Antioxidant defense, Erythrocyte indices, Thiobarbituric acid reactive substances.

Introduction

Strenuous physical exercise results in an enhanced uptake of oxygen, leading to increased metabolism, which in turn increases the production of active oxygen radical species produced by the electron transport system (1). Active oxygen species, including hydroxyl radicals, superoxide radicals, hydroperoxides, and aldehydes, are known to be toxic, mutagenic, and carcinogenic to cells (2, 3). In spite of the deleterious effects of free radicals (FRs), their benefits are now being recognised (4). FRs can abstract hydrogen from a wide range of biomolecules including the polyunsaturated fatty acids of cell membranes (5). Without the intervention of the cell's antioxidant defense mechanisms, free radical-mediated lipid peroxidation can lead to the loss of integrity of cell membranes and to tissue damage (6).

The efficiency of the antioxidant defense system depends on adequate dietary vitamin and micronutrient intake and on endogenous production of antioxidants such as glutathione (GSH) (4, 7). Recent findings indicate that exhaustive exercise in rats leads to the depletion of glutathione levels and the training programme, involving treadmill running, demonstrated increased oxidative capacity (8, 9).

The aerobic metabolic rate may increase as much as tenfold during physical exercise, enhancing leakege of O_2 from the mitochondria to the cytosol (10). The rise in oxygen free radical concentrations could exceed the protective capacity of cell antioxidant defense systems, and animal studies have shown that strenuous exercise promotes free radical formation and lipid peroxidation in erythrocyte and skeletal muscle (11, 12). It was found that training caused no apparent oxidant stress or muscle damage in rowers (13). In fact, there are many conflicting reports concerning exercise induced lipid peroxidation and changes in blood antioxidant defense systems (8, 9, 13).

In humans, evidence for free radical-mediated damage

Table 1. Physical characteristics and race completion times of participants in the half-marathon.

Subjects	Age(years)	Heigh(cm)	Weight(kg)	Race time(min)
1	24	172	59	79
2	23	187	75	76
3	22	178	70	74
4	22	173	61	72
5	25	174	67	75
6	23	179	71	77
7	21	182	73	73
8	22	171	66	74
9	26	176	67	72
10	25	170	61	71
Mean	23.3	176.2	67.0	74.3
SEM	0.52	1.70	1.71	0.79

Table 2. Hematological indices before and after the half-marathon.

PARAMETERS	PRE-RACE (median)	POST-RACE (median)
RBC(x10^{12}/liter)	4.96	5.22*
Htc (%)	44.85	45.60*
Hgb (g/dl)	14.65	15.75**
MCV (fl)	85.50	86.00
MCHC (g/dl)	34.60	34.85
WBC (x10^{9}/liter)	6.90	9.25***

Note: The statistical comparision is between pre- and post-race values; ***p<0.001, **p<0.01, *p<0.05. Others not significant (p>0.05).

Table 3. Plasma concentrations of antioxidants, total cholesterol, triglyceride, total protein and non-specific index of muscle damage and lipid peroxidation before and after the half-marathon.

PARAMETERS	PRE-RACE (median)	POST-RACE (median)
α-tocopherol (μg/ml)	7.30	7.55
Uric acid (mg/dl)	3.65	4.42*
Cholesterol (mg/dl)	119.06	133.20**
Triglyceride (mg/dl)	137.53	143.05
Total Protein (g/dl)	7.65	7.70
CK (IU/L)	137.00	149.50*
TBARS (nMol/ml)	1.71	1.80

Note: Statistical analysis and significance levels as in Table 2. Abbreviations used; CK, creatine kinase; TBARS, thiobarbituric acid reactive substances.

following exercise is less clear (14). Accordingly, the purpose of this study was to determine whether running a half-marathon would cause oxidative damage and changes in the blood antioxidant status and lipid peroxidation in athletes.

Methods

Subjects and sampling procedures: The subjects in this study were 10 healthy young males who undertook regular running exercise and competed in a half-marathon (20 km) in spring. This study was approved by the Ethics Committee of Dicle University, and all the subjects signed an informed consent document prior to taking part in the study. Physical characteristics of the subjects and the race completion times are shown in Table 1. The subjects were required to refrain from any strenuous exercise for 2-3 days prior to the race and none of the subjects took any form of medication, including anti-inflammatory agents, before the study. Exercise was carried out in the morning before breakfast. Blood was withdrawn with a syringe without stasis from the antecubital vein of each subject and immediately tranferred to heparinized glass tubes. There was no evidence of hemolysis in any of the samples. The blood samples were obtained 5 min before and after the race. All analyses were completed within a few hours of the collection of the samples.

Measurements: Superoxide dismutase (SOD) activity was measured using the method of Winterbourn et al. (15), which is based on the inhibition of the reduction of nitroblue tetrazolium by O_2 produced via photoreduction of riboflavin. 1 Unit of SOD activity was defined as fifty percent inhibition. Catalase activity was determined

Table 4. Erythrocyte antioxidant enzyme activities and glutathione before and after the half-marathon.

PARAMETERS	PRE-RACE (median)	POST-RACE (median)
CATALASE (k/gHb)	1413.0	1364.5
SOD (U/gHb)	1770.5	1710.5
TOTAL GSH (mg/gHb)	1.20	0.75*
GSH (mg/gHb)	0.85	0.68*
GSSG (mg/gHb)	0.125	0.165

Note: Statistical analysis and significance levels as in Table 2. Abbreviations used; SOD, superoxide dismutase; Total GSH, total glutathine; GSH, reduced glutathione; GSSG, oxidized glutathione.

according to the method of Aebi (16). Previously documented methods were used for the concentration of reduced and oxidized glutathione (17). The serum vitamin E concentration was determined by high pressure liquid chromatography (HPLC) (18). The lipid peroxide concentration was measured as the total thiobarbituric acid reactive substances (TBARS) as described by Asakawa and Matsushita (19). Plasma total cholesterol, total protein, creatine kinase, and uric acid concentrations were measured using an Abbott Spectrum autoanalyzer. Erythrocyte (RBC) and leucocyte (WBC) counts, hemoglobin (Hb), hematocrit (Hct), the mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) were analyzed with a (Cell-dyn, 1600) Coulter Counter.

Statistical Analysis: The Mann-Whitney U test was used to analyze the differences between the pre- and post-race values.

Results

Hematology: Immediately after the race, the RBC counts, hematocrit, total Hb and WBC counts significantly increased when compared with the pre-race values (Table 2). These changes were not due to significant alterations in the size or hemoglobin content of the red cells but were accompanied by similar and significant changes in the packed cell volume.

Plasma lipid, protein, and antioxidants: There were no significant differences in α-tocopherol, triglyceride, and total protein concentrations in terms of pre- and post-race values. However, when compared with the pre-race values, uric acid ($p<0.05$) and cholesterol ($p<0.01$) rose significantly immediately after the race, as shown in Table 3.

Plasma CK activity and index of lipid peroxidation: Plasma CK activity increased ($p<0.05$) significantly after the race. This change was not accompanied by increases in the nonspecific index of lipid peroxidation, TBARS, which remained unchanged after the race (Table 3).

Antioxidant enzyme activities and glutathione: As shown in Table 4, SOD and catalase activities did not differ significantly after the half-marathon. However, the significant decrease in total glutathione observed after the race ($p<0.05$) was mainly due to loss of the reduced form, whereas oxidized glutathione did not differ significantly from the pre-race values.

Discussion

Enhanced oxygen consumption during exercise causes mitochondrial superoxide production (10) and any resulting enhanced leakage of superoxide could promote lipid peroxidation and tissue damage. The post-race rise in plasma creatine kinase activity in this study is similar to that reported following a variety of exercise regimens (20, 21) and suggests that the running of a half-marathon induces a degree of muscle damage. However, changes in CK were not accompanied by significant post-race increases in plasma TBARS, possibly indicating that any oxidative stress incurred was insufficient to cause significant free radical-mediated peroxidation of the fatty acid components of cell membranes. Maughan et al. (22), found no changes in serum TBARS concentration or in CK ativity immediately following exercise. Six hours later, however, the serum activity of CK and the TBARS concentration were substantially higher than the pre-exercise values. This may indicate an association between the initiation of free radical reactions and the loss of membrane integrity responsible for the release of muscle-derived enzymes.

There were significant increases in RBC count, Hb concentration and packed cell volume with a resultant reduction in plasma volume after the race. These changes were not due to significant changes in the size or Hb content of the red cells but were accompanied by similar and significant changes in Hct. The increase in the WBC count after the laf-marathon was the result of exercise.

In the present study, the total protein concentration did not differ significantly after the race. In accordance with the present result, Viru and Körge (23), had earlier reported no change in plasma protein concentrations during marathon running. However, Wells et al. (24), and Maughan et al. (25), observed an increase in plasma protein concentrations following a marathon race. From

this, it is apparent that a movement of proteins into the vascular space occurred. In contrast, Dill and Costill (26), reported that protein was lost from the circulation during prolonged running. From the conflicting nature of these results, it seems probable that variations in environmental conditions, such as temperature and humidity, and differences in the peripheral circulatory response of the subjects may be important contributory factors to the differences recorded in different studies.

Plasma cholesterol and uric acid levels increased significantly after the race. These findings indicated that higher plasma cholesterol protects against erythrocyte peroxidant stress, in accordance with the observations of Bereza et al. (27).

Of the various antioxidant defense mechanisms, superoxide dismutases are the first line of defense against oxidative damage. The superoxide radicals are either dismutated by SOD or converted into hydrogen peroxide (H_2O_2). The H_2O_2 ise reduced by catalase and glutathione peroxidase (8).

Regularly performed exercise might induce an adaptive enhancement in skeletal muscle and the erythrocyte of the defense mechanisms that protect against free radical damage in athletes (12, 28). In the present study, there was no increase in erythrocyte SOD or catalase activity immediately after the race. These results were similar to those of Hugishi et al. (28) and Duthie et al. (21), but were different from the results of Vani et al. (8).

We found that athletes performing exercises regularly exhibited significant decreases in total glutathione and reduced glutathione ($p<0.05$) after the race. These results were in agreement with those of Duthie et al. (21), and Pyke et al. (29). Such results could be interpreted as indicating depletion of the glutathione antioxidant system during running exercise, leading to exhaustion. However, Lew et al. (30) reported that rats exercised to exhaustion appeared to have large increases in the total GSH and GSSG in plasma after exercise. Oxidation of GSH during submaximal exercise suggests an increase in the formation of active oxygen species in the blood (14).

The results of the present study suggest that, when trained athletes run a half-marathon, changes in erythrocyte antioxidant status may occur immediately after the race, even though the plasma index of lipid peroxidation is unaffected.

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