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Comparison of the Blood Redox Status Between Long-Distance and Short-Distance Runners

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Summary

Exercise increases the production of reactive oxygen species, which may damage a number of cell constituents. Organisms have developed a sophisticated antioxidant system for protection against reactive oxygen species. Our aim was to compare the adaptive responses of antioxidant mechanisms and the blood redox status of two groups of athletes, long-distance and short-distance runners. Thiobarbituric acid reactive substances, catalase activity and total antioxidant capacity was measured in the serum, while reduced and oxidized glutathione as well as their ratio were determined in blood hemolysates. Serum catalase activity (P<0.001) was found to be three times higher in long-distance compared to short-distance runners (25.4 vs. 8.9 μmol · min^{-1} · ml^{-1}), whereas the two groups did not differ in the other markers. Catalase activity also correlated significantly with maximal oxygen consumption in long-distance runners. In conclusion, we report here that long-distance and short-distance runners exhibit similar blood redox status judged by several oxidative stress indices, except for the much higher activity of catalase in long-distance runners. This different effect of the two training modules on catalase activity of long-distance runners might be partly due to the high oxygen load imposed during their repeated prolonged exercise bouts.

Key words

Free radicals • Lipid peroxidation • Reactive oxygen species • Total antioxidant capacity • TBARS

Introduction

Reactive oxygen species (ROS) are continuously generated in cells by a number of metabolic processes. Macromolecules, like DNA, proteins and polyunsaturated fatty acids are considered as potential targets for ROS (Halliwell 2001). Organisms have developed a sophisticated antioxidant system that protects them adequately from the hazardous effects of ROS. Antioxidants include both enzymatic molecules (eg, catalase) and non-enzymatic molecules such as glutathione (Mylonas and Kouretas 1999).

Superoxide dismutase is the first line of enzymatic defense. It converts the superoxide radical ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2): $2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$. H_2O_2, although not especially reactive, it is still

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harmful to cells and can be metabolized in a variety of ways:

a) it can be converted to H_2O by glutathione peroxidase with the consumption of reduced glutathione (GSH) which is converted to oxidized glutathione (GSSG). GSSG may be reduced back to GSH by the action of glutathione reductase at the expense of NADPH: $H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$.

b) When H_2O_2 production exceeds the capacity of glutathione peroxidase, catalase takes over the role (Urso and Clarkson 2003). It converts H_2O_2 to H_2O without the use of other substrates: $2H_2O_2 \rightarrow 2H_2O + O_2$.

c) In the presence of transition metals, like Fe^{2+} and Cu^{+}, H_2O_2 can be decomposed to produce the hydroxyl radical ($OH^{\bullet}$; i.e., the Fenton reaction), which is an extremely potent oxidizing agent that reacts with all biological macromolecules at diffusion-controlled rates (Mylonas and Kouretas 1999): $H_2O_2 + Fe^{2+}$ (or Cu^{+}) $\rightarrow OH^{\bullet} + OH^{-} + Fe^{3+}$ (Cu^{2+}). $OH^{\bullet}$ can lead to DNA damage, protein thiol oxidation and lipid peroxidation. Lipid peroxides may be further broken down to malondialdehyde (Mylonas and Kouretas 1999).

Acute exercise can produce an imbalance between ROS and antioxidants which is referred to as oxidative stress (Urso and Clarkson 2003). Several studies have generally found that chronic exercise provokes an increase in enzymatic and non-enzymatic antioxidant defense (for review see Ji 1999). This adaptation in response to training apparently serves the organism's need to enhance protection against ROS.

Despite the wealth of information regarding the effects of acute and chronic exercise on redox status, to the best of our knowledge, only one study (Marzatico *et al.* 1997) has compared the redox status of athletes of different sport disciplines. This is surprising because the effects of acute exercise on oxidative stress have been reported to be dependent on the type of exercise (Bloomer *et al.* 1995, Ilhan *et al.* 2004), which strongly implies that chronic exercise with different characteristics might provoke different adaptations in the redox system of athletes. Therefore, the aim of the present study was to compare the blood redox status of regular aerobically trained athletes (long-distance runners) and anaerobically trained athletes (short-distance runners).

Methods

Subjects

Thirty male athletes (long-distance runners n=16 and short-distance runners n=14), volunteered to participate in the present study. Subjects were not receiving anti-inflammatory medication or nutritional supplements. Athletes belong to Greek track and field clubs, and had been training for at least 6 years, not less than five times per week, at least 1 h per session. Athletes were regular participants in regional and international competitions. A written informed consent to participate in the study was provided by all participants after the volunteers had been informed of all risks, discomforts and benefits involved in the study. The procedures were in accordance with the Helsinki declaration of 1975 and approval was received for this study from Institutional Review Board.

Anthropometric and maximal oxygen consumption measurements

Subjects abstained from exercise training for 48 h prior to their visit to the laboratory. Each participant reported to the laboratory at 09:00 h after an overnight fast. After sitting for 5 min, a blood sample was withdrawn from a forearm vein. The blood was treated as described under "Collection of serum and whole blood lysate". Body mass was measured to the nearest 0.5 kg (Beam Balance 710, Seca, UK) with subjects lightly dressed and barefooted. Standing height was measured to the nearest 0.5 cm (Stadiometer 208, Seca, UK). Percentage body fat was calculated from seven skinfold measurements (average of two measurements at each site) using a Harpenden calliper (John Bull, UK), according to published guidelines (American College of Sports Medicine, 2000).

Maximal oxygen consumption (VO_2max) was determined through a maximal oxygen consumption test on a GXC200 treadmill (Powerjog, UK). The criteria for attainment of VO_2max were obtained according to those set by the American College of Sports Medicine (2000). The size, age, anthropometric, training and physiological characteristics of the subjects are shown in Table 1.

Dietary analysis

To establish that participants of both groups had similar levels of macronutrient and antioxidant intake, they were asked to follow their usual eating habits and to record their diet for three days before the blood sampling. Each subject had been provided with a written set of guidelines for monitoring dietary consumption and a record sheet for recording food intake. Diet records were analyzed using the computerized nutritional analysis

Table 1. Size, age, anthropometric and training characteristics of the participants (mean ± S.D.)

Variable	Long-distance $n = 16$	Short-distance $n = 14$
Age (years)	27.1 ± 6.9	24.9 ± 3.3
Height (m)	1.73 ± 0.08	1.80 ± 0.08*
Weight (kg)	63.6 ± 11.3	73.9 ± 6.2*
Body fat (%)	9.5 ± 4.2	7.7 ± 2.7
Years of training	10.5 ± 3.4	7.8 ± 2.9
Training sessions/week	5.4 ± 1.2	5.9 ± 1.6
VO_2max (ml/kg/min)	65.7 ± 7.2	52.0 ± 4.3*

VO_2max, maximal oxygen consumption. * Significantly different compared to long-distance runners ($P<0.05$)

Table 2. Nutritional intake of long-distance and short-distance runners (mean ± S.D.)

Variable	Long-distance	Short-distance
Energy (kcal)	2998 ± 514	3402 ± 564
Carbohydrates (% energy)	59.2 ± 14.2	53.6 ± 11.3
Fat (% energy)	31.1 ± 9.2	29.1 ± 13.2
Proteins (% energy)	1 0.1 ± 2.3	17.5 ± 3.1
Vitamin E (mg, α-TE[†])	9.4 ± 4.8	10.4 ± 3.9
Vitamin C (mg)	171 ± 116	149 ± 101
Vitamin A (mg, RE)[#]	1132 ± 495	1075 ± 517
Selenium (mg)	0.14 ± 0.04	0.13 ± 0.05
Iron (mg)	20.1 ± 4.3	19.5 ± 3.1

[#]RE, retinol equivalents. [†]alpha-TE, alpha-tocopherol equivalents

system Science Fit Diet 200A (Sciencefit, Greece). Analysis of dietary intake appears in Table 2.

Collection of serum and whole blood lysate

Venous blood from each participant was collected in vacutainer tubes. An aliquot of each sample was immediately mixed with EDTA to prevent clotting for hematology. Another aliquot of the sample was left on ice for 20 min to clot, and centrifuged at 1500 × *g* for 10 min at 4 °C in order to separate the serum. In the rest of the sample, 5 % trichloroacetic acid was added (1:1 v/v) to produce whole blood lysates, vortexed vigorously and centrifuged at 4000 × *g* for 20 min at 4 °C. The clear supernatants were transferred in Eppendorf tubes and were used for GSH and GSSG determination. All analyses were performed on the day of blood collection.

Assays

Hematocrit and hemoglobin were measured in a Sysmex K-1000 autoanalyzer (Kobe, Japan). Iron was measured spectrophotometrically with a reagent kit from Biosis (Athens, Greece), whereas ferritin was determined by enzyme immunoassay kit from DRG (Marburg, Athens). The intra-assay coefficient of variation for iron was 2.3 % and for ferritin 6.0 %.

GSH was measured according to the method of Reddy *et al.* (2004) and GSSG was determined according to the recycling method of Tietze (1969). The intra-assay coefficient of variation for GSH was 5.9 % and for GSSG 6.4 %.

Total antioxidant capacity (TAC) of the serum was based on the scavenging of 1,1-diphenyl-2-picrylhydrazyl and was determined according to Janaszewska and Bartosz (2002). Thiobarbituric acid-reactive substances (TBARS) were measured by to the method of Keles *et al.* (2001). Catalase activity was measured according to Aebi (1984). The intra-assay coefficient of variation for each assay was: TAC 4.9 %, TBARS 5.3 % and catalase 4.0 %. All assays were determined at least in triplicates.

Statistical analysis

Data are presented as mean ± S.D. The distribution of all dependent variables was examined by the Kolmogorov–Smirnov test and was found not to differ significantly from normal. Differences between long-distance and short-distance runners were examined by unpaired Student's *t*-test. Correlations between variables were examined by Pearson's correlation analysis. The level of statistical significance was set at $\alpha = 0.05$. The SPSS version 12.0 was used for all analyses (SPSS Inc., USA).

Results

Long-distance runners had significantly higher VO_2max (by 26 %) compared to short-distance runners. Hematocrit (44.2±3.3 vs 43.9±2.3), hemoglobin (14.9±1.1 vs 14.9±0.8), serum iron (107.5±24.7 vs

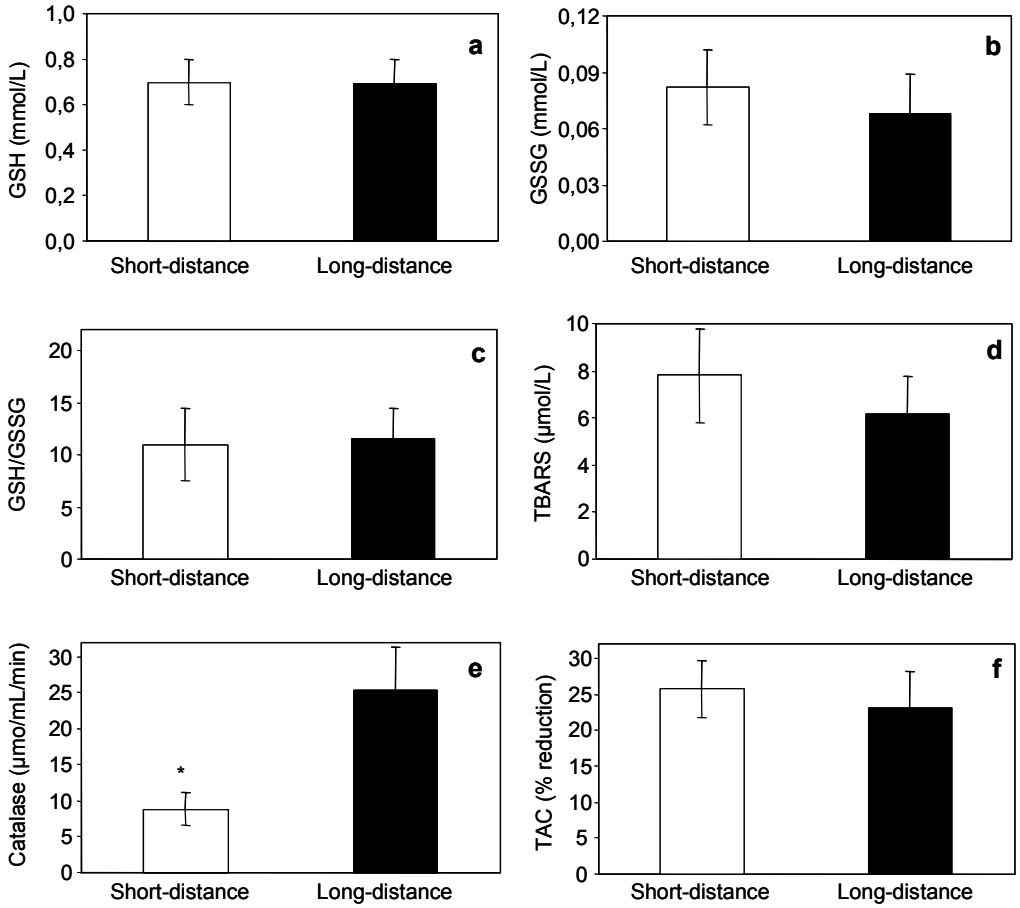

Fig. 1. Comparison of the levels of GSH (a), GSSG (b), their ratio (c), TBARS (d), catalase (e) and TAC (f) in long-distance and short-distance runners (mean ± S.D.). *Significantly different from long-distance runners (P<0.001). GSH, reduced glutathione; GSSG, oxidized glutathione; TAC, total antioxidant capacity; TBARS, thiobarbituric acid-reactive substances.

89.6±15.2) and serum ferritin (90.2±16.7 vs 59.6±10.1) concentrations did not differ between long-distance and short-distance runners. The values of redox status indices are presented in Figure 1. Serum catalase activity was found to be about three times higher in long-distance than in short-distance runners (P<0.001), whereas no significant differences were found in any biochemical parameter between the two groups. Correlations between pairs of parameters in long-distance and short-distance runners are presented in Tables 3 and 4, respectively.

Discussion

Exhausting aerobic exercise increases the oxygen demand 10- to15-fold times compared to rest, resulting in increased oxygen consumption, increased oxygen cell uptake and increased flow in the electron transport chain (Urso and Clarkson 2003). This leads to an increased release of ROS, mainly in the muscle but also in erythrocytes. Anaerobic exercise can also lead to an increased release of ROS, probably through other mechanisms, such as xanthine oxidase activation, acidosis and catecholamine oxidation (McCord 1985, Siesjö *et al.* 1985). The present study compared several oxidative stress markers between long-distance and short-distance runners. This comparison revealed that the redox status of these two groups of athletes is similar except for the much higher catalase activity in long-distance runners. This difference was underlined by the significant positive correlation between VO_2max and catalase activity in long-distance runners.

To our knowledge, only one study (Marzatico *et al.* 1997) has compared the redox status of athletes of different sports. In that study, malondialdehyde levels and the activity of three antioxidant enzymes of marathon runners were compared to that of sprinters. Marathon runners had two-fold higher values of catalase activity compared to sprinters, despite the fact that malondialdehyde levels and glutathione peroxidase activity were similar in the two groups. This is in accordance with our

findings, that long-distance runners exhibit about three times higher catalase activity than short-distance runners and no differences in TBARS.

Where does increased activity of serum catalase activity in long-distance runners come from? The most obvious answer is that it is the cumulative result of the regularly repeated aerobic exercise bouts. Indeed, based on a rather limited data, an acute bout of aerobic exercise has been reported to increase catalase activity in the blood (Marzatico *et al.* 1997, Tauler *et al.* 1999, Aguiló *et al.* 2005) even though such an effect was not found in other studies (Rokitzki *et al.* 1994, Tauler *et al.* 2003). We believe that this large increase of catalase activity in long-distance runners is at least partly resulting from a higher aerobic load imposed on those athletes due to the nature of their sport (i.e. prolonged periods of exercise). Actually, despite the much greater affinity of glutathione peroxidase for H_2O_2 compared to catalase, it has been documented that catalase activity increases when glutathione peroxidase is not sufficient to scavenge the increased levels of H_2O_2 (Urso and Clarkson 2003), a situation that may arise during endurance exercise.

In the present report, except for catalase, we also measured several other indices of oxidative stress, i.e. TBARS as a marker of lipid peroxidation, GSH and GSSG as the major redox pair in cells and TAC as an indicator of the overall antioxidant capacity of the serum. All these parameters were chosen to cover as much as possible the adaptations of the blood redox balance of athletes. Nevertheless, we found no significant differences in any of these indices between the two groups of athletes. Despite the absence of any study comparing long-distance and short-distance runners with regard to these oxidative stress indices, we considered it of interest to examine how our data compare to those from longitudinal studies using chronic endurance exercise.

As far as the glutathione system is concerned, chronic endurance exercise has been reported to either increase the antioxidant capacity of blood glutathione (i.e., increasing the GSH/GSSG ratio) (Kretzschmar *et al.* 1991) or to be without effect (Tiidus *et al.* 1996). The effect of endurance training on blood TBARS concentrations has also been found to be variable, since there are some reports of higher values (Santos-Silva *et al.* 2001) or similar levels in endurance-trained humans compared to untrained individuals (Ookawara *et al.* 2003). We are not aware whether the effect of chronic endurance exercise on blood TAC has been reported in humans. It is evident that the contradictions among various studies are so numerous that they prevent to draw clear definite conclusions about the effects of exercise on these oxidative stress indices.

It can thus be concluded that long-distance and short-distance runners exhibit no differences in several oxidative stress indices of the blood, except for the much higher activity of catalase in long-distance runners. This different effect of the two training modules on catalase activity might be partly due to the high oxygen load imposed on long-distance runners during their repeated prolonged exercise bouts. Further studies are needed to investigate the overlooked effects of chronic exercise of different type on the blood redox status in humans.

Abbreviations

GSH – reduced glutathione, GSSG – oxidized glutathione, ROS – reactive oxygen species, TAC – total antioxidant capacity, TBARS – thiobarbituric acid reactive substances, VO_2max – maximal oxygen consumption, $O_2^{\bullet -}$ – superoxide radical; H_2O_2 – hydrogen peroxide, $OH^{\bullet}$, – hydroxyl radical

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