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Changes in the Blood Antioxidant Defense Capacity During a 24 Hour Run

by
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The objective of this study was to determine whether running a 24-h race would cause oxidative damage and changes in the blood antioxidant defense capacity in endurance-trained athletes. Fourteen male amateur runners (mean age 43.0±10.8 y, body weight 64.3±7.2 kg height 171±5 cm, weekly covered distance 81±43 km, training history 8±9 y) who participated in a 24-hr ultra-marathon and volunteered to give blood samples during the race were enrolled for this study. Blood samples were taken before the run, after completing the marathon distance (42.217 km), after 12 h and at the conclusion of the race.

The capacity of erythrocyte antioxidant defense system was evaluated by measuring the activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), glutathione reductase (GR), concentrations of non-enzymatic antioxidants (uric acid and glutathione-GSH), and selected biomarkers of oxidative stress (i.e., plasma level of malondialdehyde (MDA) and plasma antioxidant capacity by FRAP (''ferric-reducing ability of plasma")). Moreover, in order to elucidate between-group differences in the total capacity of the blood antioxidant defense system, an index of antioxidant potential (POTAOX) was calculated as a sum of standardized values of activities of antioxidant enzymes (SOD, CAT, GPX, GR) and non-enzymatic antioxidants (uric acid, GSH).

A progressive decline was observed in activities of SOD and CAT with the distance covered during the race, while the opposite trend was found in activities of GPX and GR that tended to increase. A significant decrease was recorded in GSH content after completing the marathon distance, which tended toward slightly higher values, without reaching the baseline level, at the finish of the race. Plasma concentration of uric acid (UA) was not significantly affected, except for the value recorded after 12 h of running that was significantly ($p<0.05$) lower, while both markers of oxidative stress (FRAP and MDA) increased significantly after completing the marathon distance. Comparison of the calculated values of the POTAOX index recorded pre-race and throughout the competition implies that the most drastic decline in the total antioxidant capacity occurred at mid-race (i.e. after 12 h of running).

Key words: *antioxidant defense, ROS, ultra-endurance*

Introduction

Heavy-endurance exercise is associated with a substantially enhanced rate of oxygen utilization and the over-production of reactive oxygen species (ROS). Exercise-induced ROS production is believed to be mainly due to electron "leakage" from the mitochondrial respiratory chain (Turrens, 2003). ROS can also derive from the activity of xanthine oxidase, the enzyme responsible for conversion of hypoxan-

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thine to uric acid, the reaction stimulated during ischemia-reperfusion events, auto-oxidation of hemoglobin or myoglobin, and oxidation of catecholamine (Finaud et al., 2006). Exercise-induced rise in ROS concentrations could exceed the protective capacity of the endogenous antioxidant defense, which may lead to tissue damage (Ji, 1999). Ultra-endurance exercise, such as a 24-h run, provides a model to study the effects of exercise-induced oxidative stress. The objective of this study was to determine whether running a 24-h race would cause oxidative damage and changes in the blood antioxidant defense capacity in endurance-trained athletes.

Methods

Subjects

Fourteen male amateur long-distance runners were recruited from participants in a 24-hr race organized within the frame of the "Jerzy Kukuczka Running Festival" by the Academy of Physical Education in Katowice. All runners were non-smokers and did not take any medication prior to or during the race. They gave their informed written consent before entering the study, the protocol of which was approved by the local Ethics Committee for human subjects. The runners were provided with carbohydrate-rich food (sandwiches, cookies, fruits) and fluids (water and sports drinks) *ad libitum* throughout the run. Anthropometric characteristics and training status of the athletes are summarized in Table 1. As indicated by their training habits, all participants were well-trained endurance athletes.

Blood sampling and biochemical analyses

Blood samples were obtained 4 h before the race (pre-race), after completion of the marathon (42.217 km) distance (post-marathon), after 12 h (post-12 h run) and immediately after finishing the race (post-24 h run). The enzymatic antioxidant defense system in erythrocytes was evaluated by measuring activities of superoxide dismutase (SOD; EC 1.15.1.1) and glutathione peroxidase (GPX; EC.1.11.1.9) using commercially available diagnostic kits (RANSOD SD125 and RANSEL RS 504, Randox U.K., respectively), catalase (CAT; EC.1.11.1 6) by the method of Aebi (1984), and glutathione reductase (GR; EC. 1.6.4.2), according to Glatzle (1970). Plasma concentration of uric acid (UA) was analyzed with a commercially available Randox kit (UA230), and plasma level of malondialdehyde (MDA), as lipid peroxidation and oxidative stress biomarker, was estimated by the thiobarbituric acid method, according to Buege and Aust (1978), with extraction of the chromogene formed with n-butanol.

Evaluation of the blood total antioxidant capacity

Antioxidant defense system in the blood to counteract damaging effects of oxidative stress is complex in nature. It includes several antioxidant enzymes, macromolecules and an array of small molecules, such as antioxidant vitamins, thiols, uric acid, etc. (Yu, 1994). Several methods have been developed to assess the total antioxidant capacity of human serum or plasma, and most of them have tested the ability of a selected compound to scavenge free radicals or to complex metal ions driving the oxidation process (Tirzitis and Bartosz , 2010). In this study, the plasma antioxidant capacity was evaluated by the method of FRAP (''ferric-reducing ability of plasma"), according to Benzie and Strain (1996), with results expressed as μM of Trolox equivalents.

Additionally, an original approach was adopted

Table 1

Minute Physical characteristics and training status of the runners

	X±SD	Median	Range
Age,years	43,0±10,5	44,5	23-58
Height, cm	171±5	171	164-183
Body mass, kg	64,3±7,2	64,0	51,9-81,1
BMI, kg/m^2	21,9±2,1	22,3	16,4-24,2
Fat, %	6,99±2,67	7,30	2,50-11,20
LBM, kg	59,7±5,7	59,2	50,3-72,7
Training history, years	7,7±8,6	5,0	2-29
Weekly distance, km	81±43	85	20-150
Yearly distance, km	2615±2077	1500	900-7500

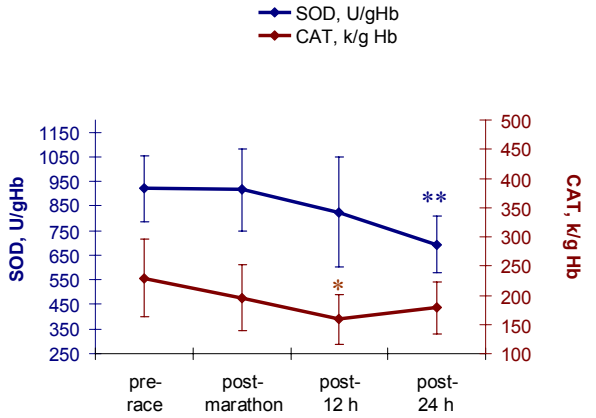

Figure 1
Changes in activities of superoxide dismutase (SOD) and catalase (CAT) during the competition

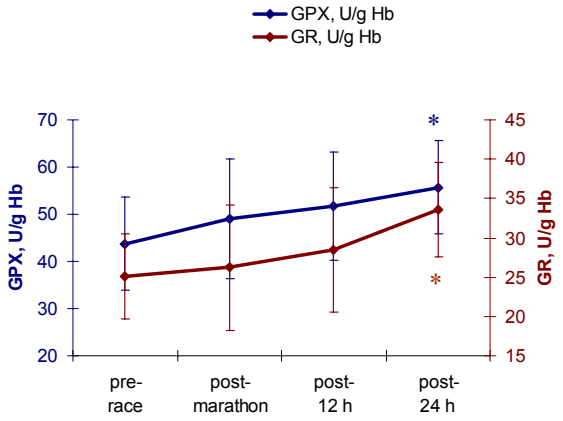

Figure 2
Changes in activities of glutathione peroxidase (GPX) and glutathione reductase (GR) during the competition

to develop a predictive model that translates individual enzymatic and non-enzymatic antioxidants measures in the blood into an index of global defense. Such an approach seems to be useful in the study targeted at evaluating differences in the response to the overall capacity of the blood antioxidant defense to external stressors, such as exercise, diet, age etc. One of the objectives of this study was to compare exercise-induced changes in the total capacity of the blood antioxidant defense system, while taking into account its individual components (i.e., activities of antioxidant enzymes (SOD, GPX, CAT, GR) and concentrations of low molecular weight antioxidants (reduced glutathione and uric acid). For this purpose, an index of antioxidant potential (POTAOX) was calculated as a sum of activities of antioxidant enzymes (SOD, CAT, GPX, GR) and non-enzymatic antioxidants (uric acid, GSH) standardized against respective pre-race values. Standardized value of a given variable (X_{iST}) means that this value is expressed in terms of its distance from the mean and SD of the reference variable (X_{ref}, SD_{ref}) recorded during pre-race, and calculated according to the formula: $xi_{ST} = (xi - X_{ref})/SD_{ref}$. The index of "total antioxidant potential" (POTAOX) was calculated using the following equation:

$$\mathbf{POTAOX=\Sigma[SOD_{ST} + GPX_{ST} + CAT_{ST} + GR_{ST} + [UA]_{ST} + [GSH]_{ST}]}$$

Statistics

The data were tested for homogeneity of variances by using the Levene test and analyzed either by the non-parametric Wilcoxon test or by one-way repeated measures ANOVA, followed, where appropriate, by the Tukey post-hoc comparisons to evaluate the effects of exercise stress. In addition, Spearman rank order correlation coefficients were calculated to assess relationships between variables. Differences were considered significant at $p<0.05$. All statistical analyses were performed using StatSoft Statistica 7.1 software.

Results

Environmental conditions at the start of the race at 8:00 pm were 11ºC and 67 % relative humidity (RH). At 8:00 am the next day the conditions were 11ºC and 71 % RH, and at the completion of the race at 8:00 pm, they were 15ºC and 55 % RH. Race performance measures are summarized in Table 2.

As expected, the highest running velocity was attained during the first stage of the race (i.e., over the marathon (42.195 km) distance); then the pace was markedly reduced throughout the remainder of the race. There were marked differences in individual race performance measures, however running velocity was not significantly related to age, body mass, BMI, or LBM. Only the relationship between running speed and body height tended towards significance (r= -0.278, p=0.075). Noteworthy, the relationship between running velocity and yearly distance covered by the athletes, which was close to significance level (r=0.310, p=0.054), may imply that

Table 2

Ultra-marathon race performance measures

	X±SD	median	Range
Marathon time, hours	4.65±0.36	4.67	4.13-5.30
Mean marathon running velocity, km/h	9.12±0.70	9.03	7.96-10.22
12-hour distance, km	94.61±11.59	94.00	73.0-109.0
Mean 12-hour running velocity, km/h	7.88±0.97*	7.83	6.08-9.08
24-hour distance, km	168.50±23.12	165.95	125.21-218.49
Mean 24-hour running velocity, km/h	7.02 ±0.96*	6.91	5.21-9.10

*- significantly different (p<0,05) from the running velocity achieved at the marathon distance

higher endurance capacity was acquired as a result of a high-volume training.

Activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR) were measured at all time points (pre-race, post-marathon, post-12 h and immediately post-race) in all runners, with data presented in Fig.1 and Fig.2. A progressive decline was observed in activities of SOD and CAT with the distance covered during the race, while the opposite trend was found in activities of GPX and GR that tended to increase. Statistical analysis of antioxidant enzyme activities revealed significant overall effect of exercise in each case ($p<0.005$ for SOD, $p<0.01$ for CAT, and $p<0.05$ for GPX and GR).

A significant decrease was recorded in reduced glutathione (GSH) content in whole blood after completing the marathon distance, which tended toward slightly higher values, without reaching the baseline level, at the finish of the race. Plasma concentration of uric acid (UA) were not significantly affected except for the value recorded after 12 h of running that was significantly ($p<0.05$) lower (Fig.3). In both cases (GSH and UA), a significant ($p<0.05$) overall effect of exercise was revealed by ANOVA. Regarding plasma antioxidant capacity, ferric reducing ability (FRAP) increased significantly ($p<0.05$) after completion of the marathon distance and stabilized at that level up to the end of the race (Fig. 4). Plasma concentration of malondialdehyde (MDA), as a biomarker of oxidative stress, increased significantly ($p<0.01$) after running the marathon distance to reach the maximum level at the completion of the race (Fig.4). Noteworthy, there was a significant ($p<0.05$) effect of exercise on both FRAP and TBARS measures. Moreover, both FRAP, as a measure of "antioxidant power" (Benzie and Strain, 1996), and MDA, as a measure of oxidative stress, were positively correlated ($r=0.302$, $p<0.05$ and $r=0.389$, $p<0.005$, respectively) with distance covered during the competition. These observation are supported by comparison of the calculated values of the POTAOX index recorded pre-race and throughout the competition (Table 3), which implies that the most drastic decline in the total blood antioxidant capacity occurred after 12 h of running.

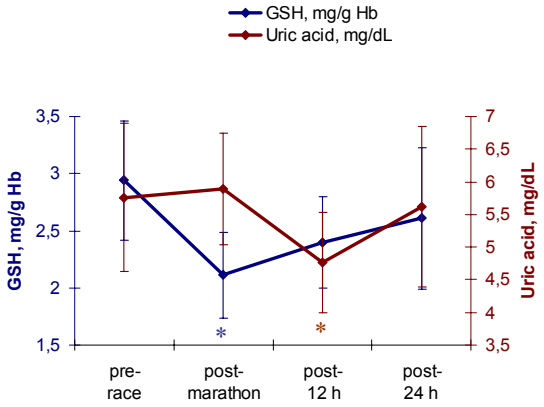

Figure 3

Changes in blood concentration of glutathione (GSH) and plasma uric acid (UA) during the competition

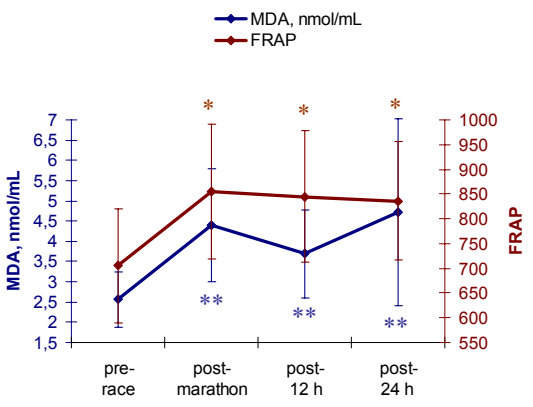

Figure 4

Changes in plasma malondialdehyde (MDA) content and Ferric Reducing Ability of Plasma (FRAP) level during the competition

Table 3
Exercise-induced changes in total antioxidant potential index (POTAOX) of blond

	Calculated POTAOX index	
	X±SEM	median
Pre-race	0.0±0.57	-0.61
Post-marathon	-1.30±0.60	-1.11
Post-12 h	-2.28*±0.65.	-3.34
Post-24 h	-0.44±0.68	-0.52

Discussion

The major finding of this study was that the ultra-endurance exercise, such as a 24 h running race, substantially increased oxidative stress and negatively affected the capacity of the antioxidant defense in the blood of all enrolled athletes. This finding is supported by consistent observations of significant decreases in activities of superoxide dismutase (SOD) and catalase (CAT). It should be stressed, however, that the activity of SOD remained practically stable until finishing the marathon distance, which decreased continuously thereafter. It is well-documented that cupro-zinc enzyme superoxide dismutase (CuZn SOD) in red blood cells (RBC) is irreversibly inactivated (up to 50 %) by its product hydrogen peroxide (H_2O_2) in a concentration-dependent manner (Salo et al., 1990). Similarly, catalase (CAT), a heme enzyme, is rapidly inhibited by superoxide anions (O_2^-) due to a reversible reaction of O_2^- radicals with native enzymes, which involves a univalent reduction of the ferric (Fe^{3+}) center to oxyferrous heme (Fe^{2+})-O_2 (Kono and Fridovich, 1982). Both enzymes, SOD and CAT, are known to act synergistically to protect erythrocytes against peroxidation (Kellogg and Fridovich, 1977), and their progressive inactivation during the competition can clearly account for substantially enhanced oxidative stress. On the other hand, a capacity to convert H_2O_2 to water and oxygen is attributed not only to CAT, but also to glutathione peroxidase (GPX), the seleno-enzyme that catalyzes the reduction of H_2O_2 and lipid peroxides to water and oxidized glutathione (GSSG), using a reduced glutathione (GSH) as the electron donor (Finaud et al., 2006). The oxidized glutathione (GSSG) is recycled back to GSH in a reaction catalyzed by glutathione reductase (GR). In the present study, activities of both enzymes (GPX and GR) tended to progressively increase, which reached statistically higher levels immediately after finishing the race. These results essentially conform to those reported by Kelle et al. (1996) after a half-marathon and by Machefer et al. (2004) after the Marathon of Sands competition, although these authors evaluated only two of the erythrocyte antioxidant enzymes (SOD and CAT or SOD and GPX, respectively).

Concerning the non-enzymatic antioxidants, this study showed significant changes in the erythrocyte GSH and plasma uric acid concentrations during the competition. Noteworthy, the concentration of GSH reached a minimum after 4.65±0.36 h (i.e., after running the marathon distance), and then it increased continuously, however, without ever reaching the baseline level at the finish of the race. Similar dynamics of blood GSH concentrations during prolonged submaximal exercise was observed by Gohil et al. (1988). These authors also found that after initial decline in blood GSH level during first 2 hours of exercise at 65 % VO_2 max, it tended to progressively increase until the end of the test, with further increases seen during recovery, but without reaching the baseline. The intensity of a 24 h race was obviously less, which implies that the dynamics of changes in GSH concentration revealed in our study could be different if comparing the time of minimum GSH level. This may also explain the differences in data presented by other authors in which a statistically significant increase in post-exercise blood GSH status was reported by Machefer et al.(2004) after Marathon des Sables, while a significant decrease was recorded by Kelle et al. (1996) after a half-marathon. According to Gohil (1988) and Sies (1985), the changes in blood GSH status after prolonged mild exercise suggest that oxidant stress may provide a signal for adaptive response of the hepatic system to stimulate GSH synthesis and efflux. This presumption has been recently supported by Lu (2009). The dynamics of changes in concentrations of uric acid was quite distinct, as the highest content of this important plasma antioxidant was recorded post-marathon, and the lowest level recorded after 12 h of running. It is postulated that urate, known as a scavenger of singlet oxygen, hydroxyl or peroxyl radicals and oxo-heme oxidants, may suppress lipid peroxidation, not only in erythrocytes, but also in longer-lived T or B lymphocytes and macrophages (Ames et al., 1981; Frei et al.,1988).

Admitting that acute bouts of endurance exercise at a moderate to high intensity increases oxidative stress, it is essential to evaluate the capacity of the

antioxidant defense system. One approach is the quantification of oxidative damage by assessment of the concentration of oxidative by-products in body fluids, tissues or expired gas (Knez et al., 2006). One of the most common measures of oxidative damage to lipids is the determination of malondialdehyde (MDA), a stable by-product derived from the oxidation of poly-unsaturated fatty acids (Nielsen et al., 1997), by the thiobarbituric acid reactive substances (TBARS) technique. Although this assay is not specific to MDA, this method is accepted as a general marker of lipid peroxidation, but results should be taken with caution (Finaud et al., 2006). In our study, the plasma MDA content, which attained the highest level at the finish of the race, is indicative of exercise-induced lipid peroxidation.

Another approach is the evaluation of the total antioxidant capacity by using selected test reactions in which total antioxidant capacity is characterized by measuring the amount of a given free radical scavenged by a test solution (e.g., blood plasma). One such method is the ferric reducing ability of plasma (FRAP) (Benzie and Strain, 1996) assay, which measures the ferric-to-ferrous iron reduction by the antioxidants present in human plasma. In the present study, FRAP level rose significantly after running the marathon distance and remained stable until the end of the competition, and its exercise-induced changes resembled those of MDA. This seems to be controversial, as a higher antioxidant capacity of plasma (evidenced by higher FRAP levels) was associated with higher level of oxidative damage to lipids (higher MDA concentrations). These discrepancies can, however, be explained by the fact that antioxidants that contribute the most to plasma FRAP assay are uric acid and ascorbic acid, while FRAP assay does not measure the contribution of glutathione (and other thiols) and serum proteins, including albumin, which are weak reductors of Fe^{3+} (Cao and Prior, 1998). Indeed, the positive relationship between FRAP and uric acid, which was near significance level ($r= 0.300$, $p=0.088$), could account for the observed response of FRAP to exercise-induced oxidative stress. Therefore, our alternative approach to use POTAOX index to quantify the blood antioxidant capacity, which yielded information about the combined effectiveness of enzymatic and non-enzymatic components of antioxidant defense system, seems to be a more reliable measure. Thus, taking pre-race condition as reference, the capacity of the blood antioxidant defense was moderately negatively affected after running the marathon distance, but was significantly reduced after 12 h, to be moderately improved at the finish of the race. It seems justified to presume that these dynamics of changes in POTAOX index reflects better the combined impact of major contributing elements to the overall blood antioxidant defense. In this particular case, a slight improvement of antioxidant capacity at the end of the race may be related to increases in activities of GPX and GR, as well as in concentrations of GSH and urate.

In conclusion, ultra-endurance exercise, such as a 24-h run, has been shown to elevate oxidative stress, as revealed by a marked decrease in activities of major antioxidant enzymes (SOD and CAT), an increase in lipid peroxidation (MDA) and a decline in the index of "total antioxidant potential" (POTAOX). Our approach to calculate POTAOX index, in order to evaluate exercise-induced changes in the capacity of the blood antioxidant defense, while taking into account the contribution of individual antioxidant species, appeared to be a useful measure of changes in the overall capacity of biological systems to withstand oxidative stress.

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