Variations in Oxidative Stress Levels in 3 Days Follow-up in Ultramarathon Mountain Race Athletes

Ypatios Spanidis,[1] Dimitrios Stagos,[1] Marina Orfanou,[1] Nikolaos Goutzourelas,[1] David Bar-or,[2,3,4,5] Demetrios Spandidos,[6] and Demetrios Kouretas[1]

[1]*Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece;* [2]*Trauma Research Department, St. Anthony Hospital, Lakewood, Colorado;* [3]*Trauma Research Department, Swedish Medical Center, Englewood, Colorado;* [4]*Trauma Research Department, Medical Center of Plano, Plano, Texas;* [5]*Luoxis Diagnostics, Inc., Englewood, Colorado; and* [6]*Laboratory of Clinical Virology, Medical School, University of Crete, Heraklion, Greece*

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Abstract

Spanidis, Y, Stagos, D, Orfanou, M, Goutzourelas, N, Bar-or, D, Spandidos, D, and Kouretas, D. Variations in oxidative stress levels in 3 days follow-up in ultramarathon mountain race athletes. *J Strength Cond Res* 31(3): 582–594, 2017—The aim of the present study was the monitoring of the redox status of runners participating in a mountain ultramarathon race of 103 km. Blood samples from 12 runners were collected prerace and 24, 48, and 72 hours postrace. The samples were analyzed by using conventional oxidative stress markers, such as protein carbonyls (CARB), thiobarbituric acid reactive substances (TBARS), total antioxidant capacity (TAC) in plasma, as well as glutathione (GSH) levels and catalase (CAT) activity in erythrocytes. In addition, 2 novel markers, the static oxidation-reduction potential marker (sORP) and the capacity oxidation-reduction potential (cORP), were measured in plasma. The results showed significant increase in sORP levels and significant decrease in cORP and GSH levels postrace compared with prerace. The other markers did not exhibit significant changes postrace compared with prerace. Furthermore, an interindividual analysis showed that in all athletes but one sORP was increased, whereas cORP was decreased. Moreover, GSH levels were decreased in all athletes at least at 2 time points postrace compared with prerace. The other markers exhibited great variations between different athletes. In conclusion, ORP and GSH markers suggested that oxidative stress has existed even 3 days post ultramarathon race. The practical applications from these results would be that the most effective markers for short-term monitoring of ultramarathon mountain race–induced oxidative stress were sORP, cORP, and GSH. Also, administration of supplements enhancing especially GSH is recommended during ultramarathon mountain races to prevent manifestation of pathological conditions.

Key Words exercise, glutathione, oxidation-reduction potential

Address correspondence to Demetrios Kouretas, dkouret@uth.gr.

31(3)/582–594

Journal of Strength and Conditioning Research

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Introduction

Ultramarathon running races have gained a lot of popularity over the last years owing to their demanding and dynamic characteristics. Actually, the same interest has been observed in the scientific field as shown by the increased number of published studies and conferences about ultrarunning worldwide. As ultramarathon is considered any sporting event involving running and walking for more than the distance of a simple marathon race (i.e., >42.195 km), these events challenge our physiological systems and can lead to significant muscle injuries, cardiac risk, inflammation, DNA, and oxidative stress damage (48,57). As a result, there is a research interest in analyzing samples from athletes participating in these running events, since they can provide useful insight into the body's responses to extreme situations.

It has been widely established in the past that intense exercise induces the production of reactive oxygen species (ROS), which may lead to a pathophysiological condition known as oxidative stress (49,76) that is associated with the oxidative damage to macromolecules (e.g., lipids, proteins, and DNA) (45), immune dysfunction (59), muscle damage (49), and fatigue (42). Reactive oxygen species are products of normal metabolism and include reactive oxygen species such as superoxide radical (o_2^-), hydroxyl radical (OH), peroxyl radical (RO_2), and reactive nitrogen species (RNS) such as nitric oxide (NO) and the peroxynitrite radical (ONOO) (24). Reactive oxygen species may participate in several cell functions such as regulation of signaling pathways and gene expression, and apoptosis (20). Reactive oxygen species endogenous sources

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include components of the mitochondrial expression, inflammatory components, peroxisomes, and cytochrome P450 activity (75). Moreover, there are also exogenous sources of ROS and RNS generation such as smoking, air pollution, ultraviolet light, and ionizing radiation (51). However, the main sources of ROS generation during strenuous exercise is the leakage of electrons from mitochondrial electron transport chain, activated neutrophils, alterations of prostanoid and catecholamine metabolism, and changes in the activity of the enzymes xanthine oxidase and nicotinamide adenine dinucleotide phosphate oxidase (14,62). These factors in combination with extramitochondrial ROS production may lead to oxidative stress (54). Several studies have reported that marathon and ultramarathon running are associated with induction of oxidative stress, since markers indicating lipid peroxidation, oxidative DNA damage, sensitivity of low density lipoprotein to oxidation and protein oxidation were increased, whereas antioxidant enzymes (e.g., superoxide dismutase) and molecules (e.g., GSH) were decreased (7,25,34,71).

As far as this study is concerned, blood samples from runners participating in the mountain ultramarathon named "Olympus Mythical Trail 2015" were used. Specifically, the aforementioned event is a mountain marathon race of 103 km, taking place in the mountain of Olympus in Greece. This race is a very demanding route that concludes with a 7,200 m elevation gain with the highest altitude of the route being at 2,906 m. Moreover, it is worth mentioning that 40 km of the route takes place at an altitude higher than 2,000 m. In this respect, previous research has documented an increase in muscle and oxidative stress levels after exercise at altitude (4). In addition, during the race the contestants must face continuous alterations between uphill and downhill routes. This is a key point as downhill running eccentrically loads the quadriceps which consequently increases myofibrillar damage that is characterized by decreased muscle force, increased serum creatine kinase (CK) activity (60,68), and inflammation response (16,36,53). During eccentric exercise, an excess amount of ROS generation occurs and has been attributed to endogenous mechanisms such as xanthine and NADPH oxidase production, ischemia reperfusion, prostanoid and catecholamine metabolism, disruption of iron-containing proteins, and excessive calcium accumulation (41). Furthermore, at the injury point, neutrophils and macrophages are infiltrated and may induce ROS production (5,10). However, an intriguing fact that has been recently reported to be happened after eccentric exercise is the variability between the individuals as many individuals exhibited reductive or negligible stress (39,66).

In a previous study, we have assessed mountain marathon–induced oxidative stress in athletes immediately postrace, using among others some novel oxidation-reduction potential (ORP) markers (65). A key finding that derived from the latter study was the huge and significant differences that were observed in almost all tested markers postrace (static ORP (sORP), GSH, CAT, TBARS, CARB, and TAC) indicating a very oxidative environment after the competition. As the analysis of the previous study based only in one time point, immediately after the competition, the aim of this study was to expand the monitoring postexercise period of ultramarathon mountain–induced oxidative stress to beyond 3 days after completion of the ultramarathon event (i.e., from 24 to 72 hours postrace). Thus, findings from this study will contribute to a better understanding of the adaptive mechanisms against oxidative stress induced by such athletic events and consequently would help to improve immediate recovery process, health status, and performance of the athletes (e.g., by altering nutrition and/or administrating antioxidant supplements). Moreover, ORP markers were again used to examine whether they are effective for monitoring ultramarathon mountain–induced oxidative stress in a short-term period. Finally, we aimed at analyzing at an individual level, the induction of oxidative stress, since as-mentioned great variability has been reported between different individuals.

METHODS

Experimental Approach to the Problem

Blood samples from 12 adult male runners participating in the mountain ultramarathon named "Olympus Mythical Trail 2015" were drawn 8 hours prerace and 24, 48, and 72 hours postrace. The determination of the athletes' redox status was based on conventional spectrophotometric oxidative stress markers such as thiobarbituric acid reactive substances (TBARS), protein carbonyl levels (CARB), total antioxidant capacity (TAC) in plasma, and glutathione (GSH) levels, and catalase (CAT) activity in red blood cells. In addition, a novel method based on the measurement of ORP using the RedoxSYS Diagnostic System (Luoxis Diagnostics, Inc., Englewood, CO, USA) was also assessed. Specifically, ORP is an integrated measure of the balance between total oxidants (e.g., oxidized thiols, superoxide radical, hydroxyl radical, hydrogen peroxide, nitric oxide, peroxynitrite, and transition metal ions) and total reductants (e.g., free thiols, ascorbate, α-tocopherol, β-carotene, and uric acid). In previous studies, we have shown that the RedoxSYS Diagnostic System is an effective, fast, and safe method for measuring ORP markers for the determination of oxidative stress induced by mountain marathon race, eccentric exercise, and after a strenuous basketball season (64–66).

Subjects

The participants were 12 adult male athletes (age 41.1 $\pm$ 3.2 years; age range 26–55 years; height 1.78 $\pm$ 0.02 m; weight 72.9 $\pm$ 2.0 kg) who voluntarily participated in the study. Written informed consent to participate in the study was provided by all athletes after they had been informed of the benefits and risks of the investigation. The procedures were in accordance with the Declaration of Helsinki of 1975 as revised in 2000, and approval was received by the human subjects committee of the University of Thessaly.

Furthermore, the subjects did not receive anti-inflammatory medicines or nutritional supplement before the competition,

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as they were informed by our team to refrain from the consumption of any kind of the aforementioned supplements. During the competition, each athlete had the ability to consume short meals including mainly carbohydrates but none of them had any additional supplementation. They were also all experienced and familiar with mountain running, as for the participation it was required at least 3 mountain marathon completed races in the last 3 calendar years before the current year registration procedure.

The participants visited the Litohoro Health Center, located close to the starting point of the race to complete a health and activity questionnaire, and their anthropometric parameters were taken. Body mass was measured to the nearest 0.5 kg (Beam Balance 710; Seca, Birmingham, United Kingdom) with the subjects lightly dressed and barefoot. Standing height was measured to the nearest 0.5 cm (Stadiometer 208; Seca).

Snapshot of the Competition

This study was performed at one of the most extreme mountain ultramarathons worldwide named "Olympus Mythical Trail", organized on July 4–5, 2015 in Olympus Mountain in Greece. The route distance was 103 km, whereas the total ascent (i.e., positive height difference) reached 7,200 m (more than 2 times the altitude of Olympus Mountain). It was a "loop" course type and consisted mostly of paths (95%) and dirt (5%), starting and ending at Litochoro, a town in northern Greece.

Moreover, the route consisted of 18 checkpoints while about 40 km of it passed above 2,000 m altitude. The maximum time allowed for race completion was 28 hours.

Procedures

Blood Collection and Assays. The blood samples were collected at 4 different time points: 8 hours prerace and 24, 48, and 72 hours postrace. The samples (10 ml) were drawn from a forearm vein with subjects in seated position, stored in EDTA or heparin tubes and centrifuged at 1,370*g* for 10 minutes at 4°C to divide the erythrocytes from the plasma. The packed erythrocytes were lysed with 1:1 (vol/vol) distilled water, inverted vigorously, and centrifuged at 4,020*g* for 15 minutes at 4°C. The plasma and erythrocyte lysates were then stored at −80°C before biochemical analysis.

The samples stored in heparin tubes were used for the determination of ORP markers by using the RedoxSYS Diagnostic System (Luoxis Diagnostics, Inc.) as described previously (64–66). The RedoxSYS system consists of a small battery-powered reader and small sensors that require limited sample manipulation, as it measures ORP within 4 minutes in 20 μl of heparinized mammalian plasma samples. Specifically, the biological sample is applied to a RedoxSYS sensor inserted into a galvanostat-based reader. The test starts when the sample fills the reference electrode, thereby completing the electrochemical circuit. Then, sORP is recorded in mV and displayed on the RedoxSYS display screen. Namely, sORP is the standard potential between a working electrode and a reference electrode with no driving current (or extremely small current), which is proportional to the balance of reductants and oxidants and is what is classically termed ORP. Low sORP values (<150 mV) mean that the biological sample is in the normal range of oxidative stress, whereas higher than normal sORP values (>150 mV) means that

Table 1. Depiction of each athlete's performance.*

Individual	Result	Performance
1	Finished	19 h 37 min
2	Finished	27 h 26 min
3	Finished	22 h 30 min
4	Finished	20 h 19 min
5	Finished	20 h 27 min
6	Finished	23 h 39 min
7	DNF (70th km)	15 h 29 min
8	DNF (60th km)	15 h 01 min
9	Finished	27 h 58 min
10	DNF (70th km)	18 h 03 min
11	Finished	21 h 25 min
12	DNF (60th km)	14 h 57 min

*For athletes who did not finish (DNF), the kilometer at which they quit is also displayed.

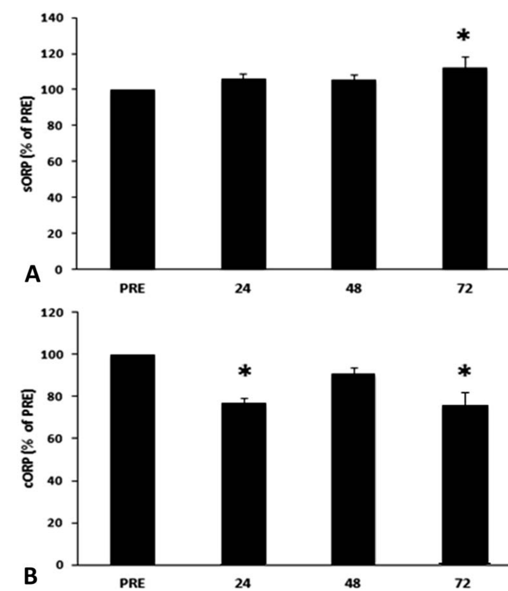

Figure 1. Changes (%) of static oxidation-reduction potential (sORP) (A) and capacity oxidation-reduction potential (cORP) (B) markers, measured in plasma at 24, 48 and 72 hours postrace compared with prerace. All values are presented as the mean ± SEM. *Significantly different compared with prerace ($p < 0.05$).

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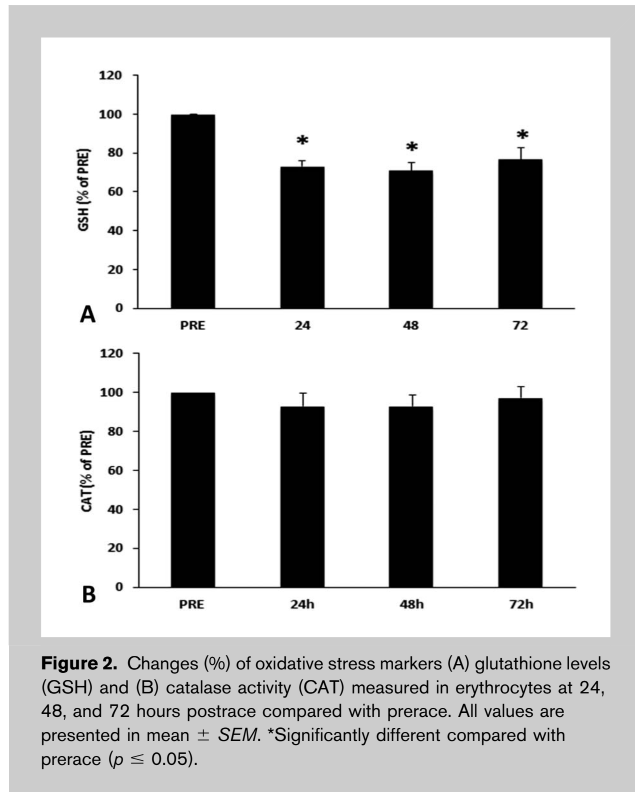

Figure 2. Changes (%) of oxidative stress markers (A) glutathione levels (GSH) and (B) catalase activity (CAT) measured in erythrocytes at 24, 48, and 72 hours postrace compared with prerace. All values are presented in mean ± *SEM*. *Significantly different compared with prerace ($p \leq 0.05$).

the biological sample is in a higher state of oxidative stress. After sORP measurement, the reader applies a small current sweep to the sample, resulting in the exhaustion of antioxidant species. As a result, the capacity ORP (cORP) of the sample is estimated in microcoulombs and reported on the RedoxSYS display screen. That is, cORP reflects the amount of electrons applied to the sample that causes the exhaustion of antioxidants in the sample. Capacity ORP is the measure of antioxidant reserve available in the body's system. High cORP values mean that the biological sample has antioxidant reserves in the normal range, whereas lower than normal cORP values means that the biological sample has below normal antioxidant reserves.

For TBARS determination, the assay was based on Keles et al. (32). TBARS is a widely and frequently used method to determine lipid peroxidation. According to the method, 100 μl of plasma was mixed with 500 μl of 35% trichloroacetic acid (TCA) (Merck, Darmstadt, Germany) and 500 μl of Tris–HCl (Sigma-Aldrich, St. Louis, MO, USA) (200 mM, pH 7.4) and incubated for 10 minutes at room temperature. One milliliter of 2 M sodium sulfate (Na_2SO_4) and 55 mM thiobarbituric acid (TBA) solution were added and the samples were incubated at 95°C for 45 minutes. The samples were cooled on ice for 5 minutes and vortexed after adding 1 ml of 70% TCA. The samples were centrifuged at 15,000*g* for 3 minutes, and the absorbance of the supernatant was read at 530 nm using a spectrophotometer (Hitachi U-1900; serial no. 2023-029; Hitachi, Tokyo, Japan). A baseline absorbance

Table 2. GSH values ($\mu mol \cdot g^{-1}$ Hb) of the participants at all time points (pre-race, 24 hours, 48 hours, 72 hours postrace).

Individual	GSH ($\mu mol \cdot g^{-1}$ Hb)			
	Pre-race	24 hours	48 hours	72 hours
1	4.49	3.11	3.76	3.48
2	6.41	4.27	4.62	7.21
3	6.29	5.27	4.31	4.22
4	7.53	6.45	6.46	7.61
5	2.63	2.57	2.57	2.60
6	6.27	4.57	3.83	4.39
7	3.99	2.42	1.82	2.06
8	3.16	2.05	1.52	1.86
9	3.99	3.21	2.83	2.74
10	9.05	5.46	7.43	7.44
11	4.49	2.88	3.62	2.34
12	2.89	2.31	2.37	2.99

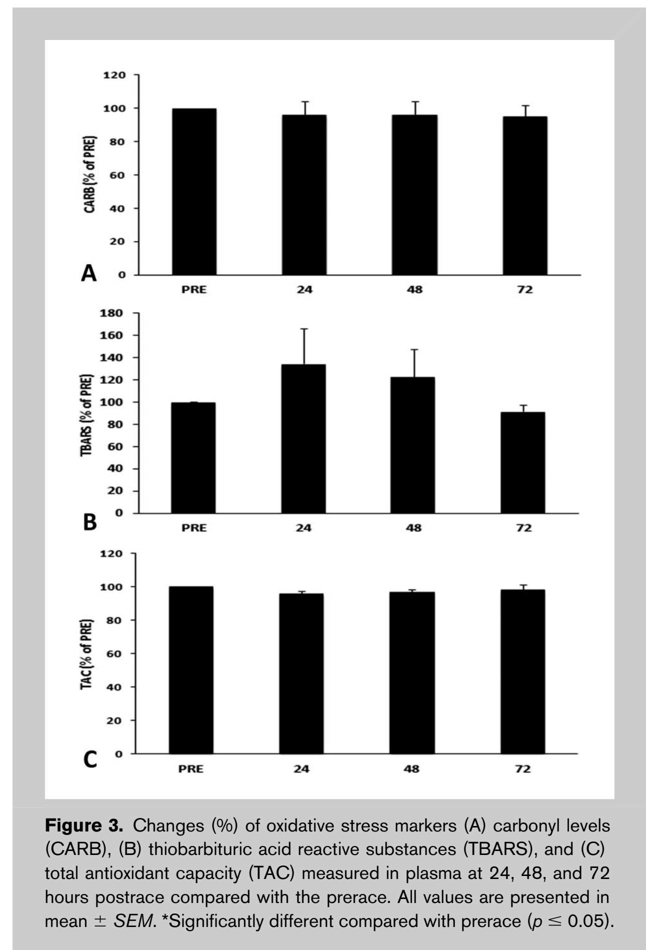

Figure 3. Changes (%) of oxidative stress markers (A) carbonyl levels (CARB), (B) thiobarbituric acid reactive substances (TBARS), and (C) total antioxidant capacity (TAC) measured in plasma at 24, 48, and 72 hours postrace compared with the prerace. All values are presented in mean ± *SEM*. *Significantly different compared with prerace ($p \leq 0.05$).

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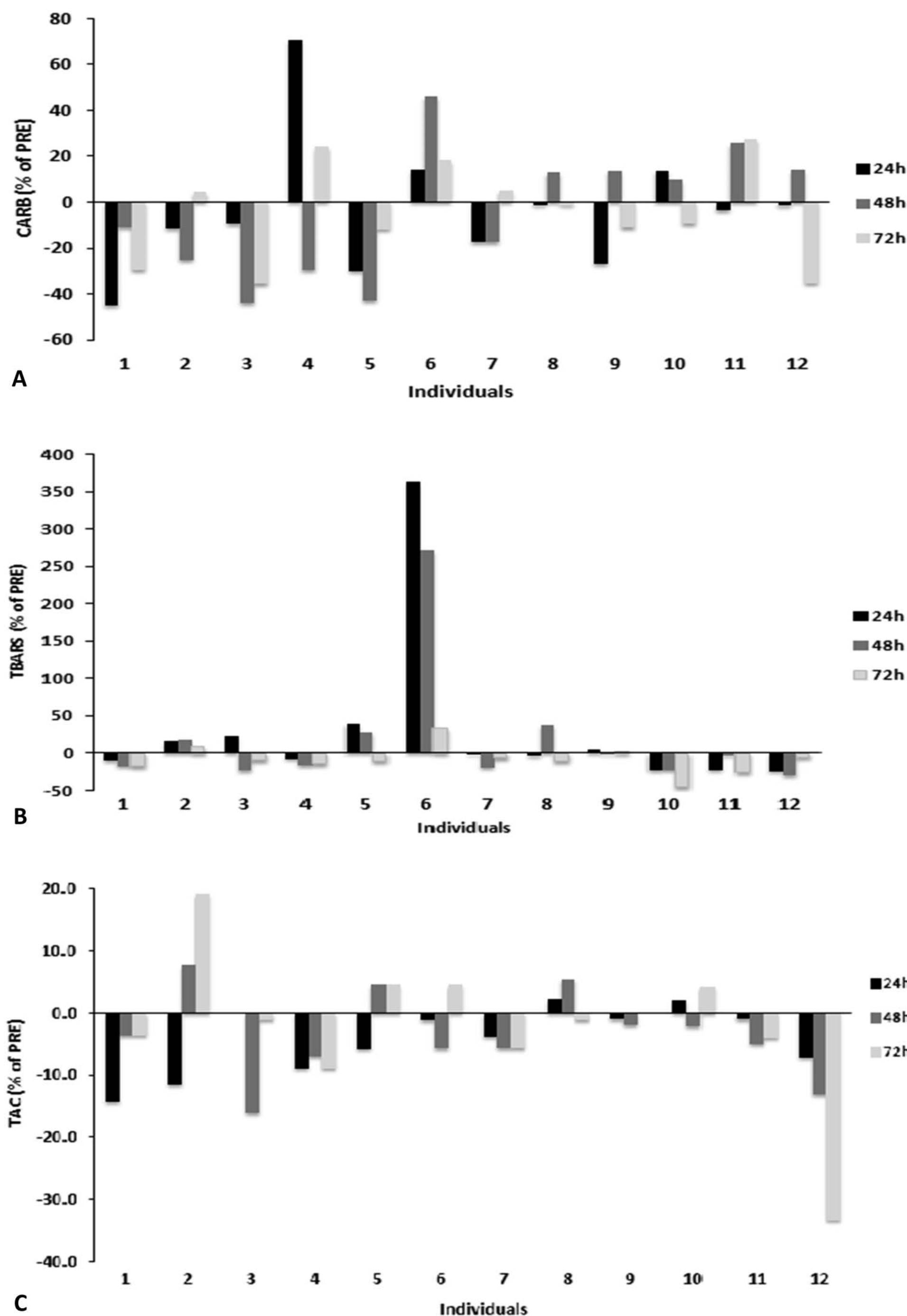

Figure 4. Changes (%) of oxidative stress biomarkers in each individual examined in plasma at 24, 48, and 72 hours postrace compared with prerace. Each bar represents the percentage difference in the level of each biomarker at postrace compared with prerace. (A) Protein carbonyls levels (CARB), (B) thiobarbituric acid reactive substances (TBARS), and (C) total antioxidant capacity (TAC).

was taken into account by running a blank (containing water instead of plasma) along with all samples during the measurement. Calculation of TBARS concentration was based on the molar extinction coefficient of malondialdehyde.

Protein carbonyls were determined based on the method of Patsoukis et al. (52). In this assay, 50 μl of 20% TCA was added to 50 μl of plasma, and this mixture was incubated in an ice bath for 15 minutes and centrifuged at 15,000*g* for 5 minutes at

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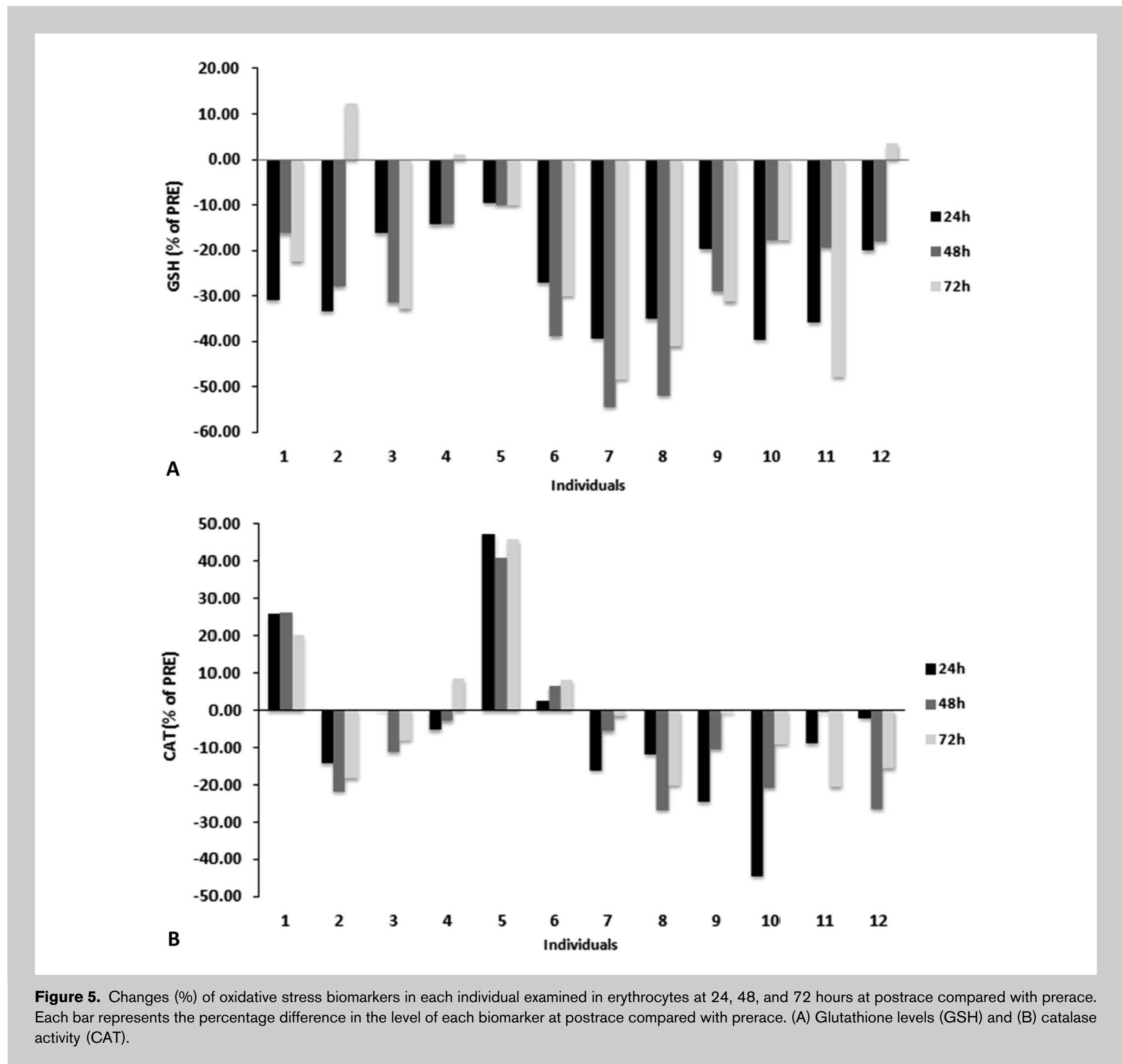

Figure 5. Changes (%) of oxidative stress biomarkers in each individual examined in erythrocytes at 24, 48, and 72 hours at postrace compared with prerace. Each bar represents the percentage difference in the level of each biomarker at postrace compared with prerace. (A) Glutathione levels (GSH) and (B) catalase activity (CAT).

4°C. The supernatant was discarded and 500 μl of 10 mM 2,4-dinitrophenylhydrazine (in 2.5 N HCl) for the sample, or 500 μl of 2.5 N HCl for the blank, was added in the pellet. The samples were incubated in the dark at room temperature for 1 hour with intermittent vortexing every 15 minutes and were centrifuged at 15,000*g* for 5 minutes at 4°C. The supernatant was discarded and 1 ml of 10% TCA was added, vortexed, and centrifuged at 15,000*g* for 5 minutes at 4°C. The supernatant was discarded and 1 ml of ethanol-ethyl acetate (1:1 vol/vol) was added, vortexed, and centrifuged at 15,000*g* for 5 minutes at 4°C. This washing step was repeated twice. The supernatant was discarded and 1 ml of 5 M urea (pH 2.3) was added, vortexed, and incubated at 37°C for 15 minutes. The samples were centrifuged at 15,000*g* for 3 minutes at 4°C, and the absorbance was read at 375 nm. Calculation of protein carbonyls concentration was based on the molar extinction coefficient of DNPH. Total plasma protein was assayed using Bradford reagent.

Moreover, the determination of TAC was based on the method of Janaszewska and Bartosz (26). In this assay, 20 μl of plasma were added to 480 μl of 10 mM sodium potassium phosphate (pH 7.4) and 500 μl of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. Then, the samples were incubated in the dark for 30 minutes at room temperature and then centrifuged for 3 minutes at 20,000*g*. The absorbance was read at 520 nm using a spectrophotometer (Hitachi U-1900; serial no. 2023-029). TAC is presented as mmol of DPPH reduced to 2,2-diphenyl-1-picrylhydrazine by the antioxidants of plasma.

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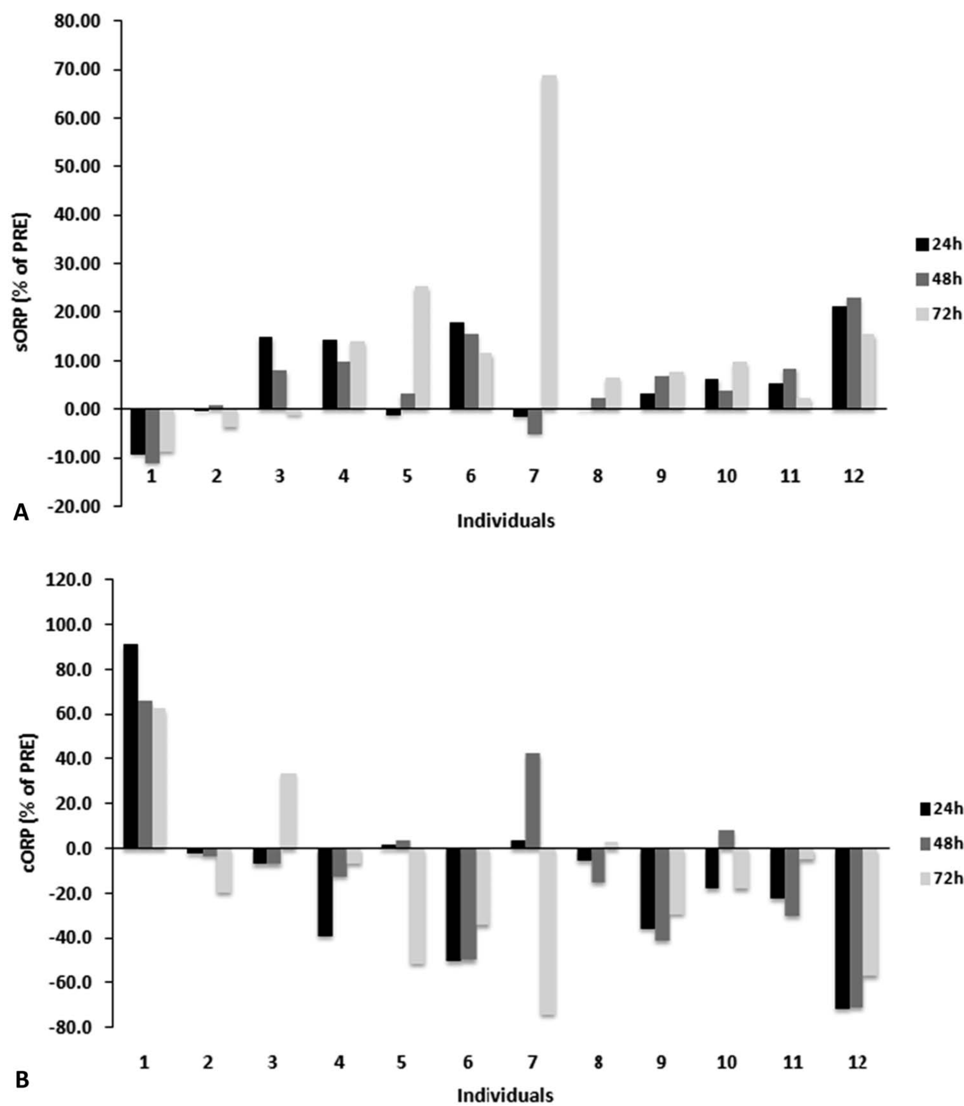

Figure 6. Changes (%) of oxidation-reduction potential (ORP) biomarkers in each individual examined in plasma at 24, 48, and 72 hours at postrace compared with prerace. Each bar represents the percentage difference in the level of each biomarker at postrace compared with prerace. (A) Static oxidation-reduction potential (sORP) and (B) capacity oxidation-reduction potential (cORP).

In addition, GSH levels were measured in red blood cells by using an assay based on the study by Reddy et al. (55). In this assay, 20 μl of erythrocyte lysate treated with 5% TCA, mixed with 660 μl of 67 mM sodium potassium phosphate (pH 8) and 330 μl of 1 mM 5, 5-dithiobis-2 nitrobenzoate (DTNB). The samples were incubated in the dark at room temperature for 45 minutes and the absorbance was read at 412 nm using a spectrophotometer (Hitachi U-1900; serial no. 2023-029). GSH concentration was calculated relative to a calibration curve made using commercial standards.

Finally, the measurement of catalase (CAT) enzyme activity was based on the method of Aebi et al. (1). Specifically, 4 μl of erythrocyte lysate (diluted 1:10) was added to 2,991 μl of 67 mM sodium potassium phosphate (pH = 7.4), and the samples were incubated at 37°C for 10 minutes. Five microliters of 30% hydrogen peroxide (H_2O_2) were added to the samples and the change in absorbance was immediately read at 240 nm using a spectrophotometer (Hitachi U-1900; serial no. 2023-029) for 130 seconds. Calculation of CAT was based on the molar extinction coefficient of H_2O_2.

Statistical Analyses

The statistical analysis was based on one-way analysis of variance followed by Dunnett's test for multiple pairwise comparisons. The statistical significance level was set at $p \leq 0.05$. For all statistical analyses, SPSS version 13.0 (SPSS,

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Table 3. Comparison (p values) of the levels of oxidative stress markers between finishers (F) and nonfinishers (NF) of the ultramarathon mountain race at 24, 48, and 72 hours postrace.*

	p		
Oxidative stress markers	F-NF (24 hours)	F-NF (48 hours)	F-NF (72 hours)
CARB	0.172	0.517	0.713
TBARS	0.171	0.361	0.163
TAC	0.248	0.920	0.314
GSH	0.125	0.326	0.689
CAT	0.120	0.025†	0.097
sORP	0.890	0.909	0.286
cORP	0.806	0.615	0.671

*CARB = carbonyls; TBARS = thiobarbituric acid; TAC = total antioxidant capacity; GSH = glutathione; CAT = catalase activity; sORP = Static oxidation-reduction potential; cORP = capacity oxidation-reduction potential.
†Significantly different between F and NF ($p \leq 0.05$).

Inc., Chicago, IL, USA) was used. Data are presented as mean ± *SD*.

Results

Subject Completion

After the completion of the race, 8 of 12 participants achieved to finish the race, whereas 2 of them terminated their participation at the 70th km point and another 2 terminated their participation at the 60th km point (Table 1). The mean running time of the athletes was 19.57 ± 1.09 hours.

Results

The results showed that the sORP marker representing the current redox status increased significantly ($p \leq 0.05$) by 14.29% at 72 hours postrace compared with prerace samples, indicating an induction of oxidative stress (Figure 1A). Moreover, the cORP marker exhibited a significant decrease ($p \leq 0.05$) at 24 and 72 hours postrace by 22.26 and 23.29%, respectively, compared with prerace samples, suggesting decreased reserves of antioxidants (Figure 1B).

Regarding the "conventional" oxidative stress markers, GSH levels in erythrocytes exhibited a significant decrease ($p \leq 0.05$) both at 24, 48, and 72 hours postrace by 26.78, 29.04, and 23.19%, respectively, compared with prerace (Figure 2A). Since GSH is one of the most important antioxidant molecules of the organism, the absolute values of GSH are presented in Table 2 at prerace to be examined if high GSH values prerace provided more protection from oxidative damage than low GSH values prerace.

However, none of the other oxidative stress markers (i.e., CARB, TBARS, TAC, and CAT) exhibited a significant change ($p \leq 0.05$) at any time point postrace compared with prerace (Figures 2B and 3A–C).

We also examined the changes occurring in oxidative stress markers at all time points for each individual (Figures 4–6). It was shown that in all runners, apart from one (subject 1), the sORP values increased at least at one time point postrace compared with prerace (Figure 6A) Similarly, cORP values in all runners apart from one (subject 1) exhibited a decrease at least at one time point postrace compared with prerace (Figure 6B).

Regarding the other oxidative stress markers assessed in plasma, CARB, TBARS, and TAC exhibited great variations between the ultramarathon runners (Figure 4). Similarly, CAT values measured in erythrocytes exhibited great variation among different individuals (Figure 5B). The changes in GSH levels in erythrocytes exhibited a similar pattern between different athletes, that is, they were decreased in all runners at 24 and 48 hours postrace, and in 9 runners of 12 at 72 hours postrace compared with prerace (Figure 5A).

It is worth mentioning that the division of the athletes between finishers and nonfinishers and the respective analysis

Table 4. Mean values (±*SD*) of each oxidative stress marker at all time points (prerace and 24 hours, 48 hours, 72 hours postrace).*

	Prerace	24 hours	48 hours	72 hours
sORP (mV)	131.10 ± 8.50	138.90 ± 14.80	138.20 ± 13.50	146.90 ± 20.70
cORP (μC)	0.63 ± 0.06	0.52 ± 0.08	0.54 ± 0.07	0.49 ± 0.06
GSH ($\mu Mol \cdot g^{-1}$ Hb)	4.93 ± 0.67	3.54 ± 0.49	3.56 ± 0.57	3.91 ± 0.68
CAT ($U \cdot mg^{-1}$ Hb)	278.30 ± 20.90	271.50 ± 16.90	271.50 ± 18.90	280.30 ± 19.10
CARB ($nMol \cdot mg^{-1}$ protein)	0.52 ± 0.09	0.49 ± 0.12	0.49 ± 0.11	0.49 ± 0.12
TBARS ($\mu Mol \cdot L^{-1}$)	5.89 ± 1.46	6.77 ± 2.38	6.34 ± 2.62	5.20 ± 0.73
TAC (mmol $DPPH \cdot L^{-1}$)	0.93 ± 0.08	0.89 ± 0.11	0.89 ± 0.08	0.91 ± 0.11

*sORP = Static oxidation-reduction potential; cORP = capacity oxidation-reduction potential; GSH = glutathione; CAT = catalase activity; CARB = carbonyls; TBARS = thiobarbituric acid; TAC = total antioxidant capacity.

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has not exhibited any significant changes in any marker, apart from CAT levels at 48 hours postrace (Table 3). Moreover, the changes in the tested markers have indicated induction of oxidative stress at postrace compared with prerace in nonfinishers (Figures 4–6). Thus, the results were analyzed as a whole group and not separately as finishers and nonfinishers.

Discussion

In recent years, ultramarathons and especially mountain marathons have gained a lot of popularity because of their demanding and strenuous characteristics. Since the first ultraendurance race in the late 1970s, around 1,000 races are now held worldwide each year. The participation in such competition requires an extremely good physical condition and enough experience for every participant, as he is exposed to a huge muscle and respiratory distress. The organism's response to these strenuous situations is considered very interesting, as the human body gets pushed to its limits. Thus, there is a recent growing research interest in analyzing biological parameters from athletes participated in this kind of race (15,44,65,79). In a previous study, we have shown that mountain marathon runners exhibited significant changes in their redox status immediately postrace, especially in the novel ORP markers (65). The aim of this work was to assess the changes in the athletes' redox status the next days after the race, so as to examine the development of possible adaptive mechanisms as a response to oxidative stress. Moreover, we analyzed the interindividual variability of the response to oxidative stress.

The ultramarathon route was 103 km, with 40 km over 2,000 m altitudes. As mentioned above, the type of exercise endured by the athletes was mainly aerobic including many eccentric parts due to the downhill paths. As a result of the obvious excessive amounts of exercise and the extremely difficult effort made by every athlete, there was severe oxidative stress postrace. Specifically, the GSH levels in erythrocytes were significantly reduced at 24, 48, and 72 hours postrace compared with prerace (Table 4). This finding was in agreement with our previous study in mountain marathon runners, showing decreased GSH immediately postrace (65), as well as with other studies (17,19,72). However, Skenderi et al. (62) has reported no significant changes in GSH levels postrace compared with prerace in athletes who took part in the Spartathlon ultramarathon in Greece. However, it should be mentioned that Spartathlon race is a flat surface race in low altitude and so it does not include as much eccentric exercise. Generally, it has been proposed that the exercise-induced increase in ROS activates adaptive responses through signaling pathways regulated by the thiol status (i.e., mainly reduced and oxidized GSH levels) (27,43,80,81). Moreover, the expression of nuclear factor-κB and activator protein-1 are induced by changes in the thiol redox status and thus increasing cytokines IL-6 and TNF-α levels (27,33). It is believed that both IL-6 and TNF-α affect muscle regeneration and the development of tolerance after ROS-induced muscle damage (67).

Furthermore, significant changes were displayed both in sORP and cORP, the 2 novel markers measured by the RedoxSYS Diagnostic System. Specifically, sORP was increased at 72 hours after the competition indicating induction of oxidative stress. In previous studies, we have also shown that this marker was increased and associated with oxidative stress induction after endurance and strenuous exercise (55,65,66). Regarding cORP, a statistical significant decrease was observed at 24 and 72 hours postrace compared with prerace. As mentioned, cORP is an integrated measure of the antioxidant reserve available in the body's system. Therefore, after such a demanding exercise antioxidant reserves were decreased, as the participants were not able to cope with oxidative stress and/or to replace their antioxidant reserves after exercise. However, in one of our previous studies conducted in a mountain marathon race, no significant changes were observed in cORP immediately postrace compared with prerace (65). This contradiction between the 2 studies may be explained by the fact that blood samples were taken at different time points, that is, 24, 48, and 72 hours postrace in the present study and immediately postrace in the other study. The present finding that there may exist time dependent differences in cORP marker is quite significant and may be correlated with muscle damage caused by eccentric exercise. In particular, it is widely known that macrophages, eosinophils, and neutrophils can induce ROS production (37). These cells also present significant activation during eccentric exercise as a result of the lengthening muscle contractions (37). When oxidative stress exists, antioxidant molecules are depleted because of the cells' effort to counteract the oxidant effects and restore the redox balance by activation or silencing of genes encoding defensive enzymes, transcription factors, and structural proteins (63). This decrease in antioxidants may explain the decreased cORP levels observed several days postrace. However, since these physiological processes need several days to develop, antioxidant depletion and a subsequent decrease in cORP may take a few days to develop thus explaining the lack of change seen immediately postexercise in our previous work.

Moreover, the interindividual analysis of ORP markers showed an unexpected fact in one athlete (No. 1) who exhibited a different response to the strain of the competition than the other athletes, as his sORP values were decreased and cORP values were increased at all time points postrace compared with prerace. We have also previously shown a wide range of responses regarding cORP between different individuals after exhaustive eccentric exercise (66). Also, this athlete exhibited decreased TBARS and CARB levels postrace compared with prerace. Moreover, other studies have reported induction of reductive stress and not oxidative stress after eccentric exercise (39). A possible explanation for this observation is that this particular athlete could confront the exercise-induced oxidative stress by increasing his antioxidant reserves, leading therefore to increased cORP and decreased sORP levels.

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Unlike ORP markers, TBARS levels indicating lipid peroxidation did not exhibit significant changes the days post-exercise compared with pre-exercise. Previous studies on ultraendurance exercise have shown that TBARS was consistently higher immediately after ultramarathons and remained elevated for 24–48 hours (30,46,62,65,73). However, a previous study of our research group on eccentric exercise has shown no significant changes in TBARS postexercise compared with pre-exercise (66), similarly to many other studies on acute and aerobic exercises (2,6,47). This lack of significance regarding the changes of the lipid peroxidation after exercise can be explained by the great interindividual variation observed between participants. In particular, 6 of 12 participants exhibited an increase in TBARS levels postrace compared with prerace at least at one time point while the rest exhibited a decrease at all time points. So, it is obvious that concerning the influence of exercise on the process of lipid peroxidation, there are different results among different studies and between different individuals of the same study as well (39,40,66).

Moreover, on average, CARB levels, indicating protein oxidation, were not changed significantly postrace compared with prerace. This was in contrary with our previous study (65) and with other studies reporting increase in CARB after strenuous exercise in human and in animals (35,58). However, Bloomer et al. (6) has shown that there were no significant changes in protein oxidation levels in cyclists, 24 hours post exhaustive aerobic exercise, a type of exercise that is similar to our study. Similarly, a study on ultramarathon swimming, an exhaustive form of oxidative stress, showed no change in either CARB levels postexercise compared with pre-exercise (28). We believe that these differences between studies regarding changes in CARB levels exist because of individual variation of protein oxidation in response to oxidative stress. Specifically, 9 participants exhibited an increase postrace (at least at one time point) compared with prerace while the rest 3 participants demonstrated a decrease at all time points.

Regarding TAC, it did not differ on average significantly postrace compared with prerace. This marker also exhibited great variation between different individuals. Specifically, 5 athletes exhibited an increase especially at the 48- and 72-hour time points. Other studies have also reported increase in TAC postexercise (18,65,77). However, 7 athletes exhibited a decrease in TAC postrace compared with prerace at all time points. The decrease in TAC postrace at all time points compared with prerace may be explained by the inability of some individuals to effectively activate adaptive responses to oxidative stress induced by such a demanding exercise. Changes in TAC expected to correspond with changes seen in cORP, since both markers assess the total antioxidant capacity. However, only 4 athletes exhibited a similar pattern of changes between these 2 markers. The differences between these 2 markers may be explained by the different methodologies used in each case. TAC is based on the reduction of a free radical (i.e., DPPH) by the antioxidant molecules in plasma, whereas cORP is based on the amount of electrons needed for the exhaustion of antioxidants in the sample. The significant decrease in cORP postrace indicated induction of oxidative stress, like sORP changes. However, TAC did not change significantly postrace, indicating the absence of oxidative stress. Thus, cORP may be a more efficient marker than TAC for assessing exercise-induced antioxidant capacity particularly when one considers the other changes observed in this study.

Finally, CAT activity in erythrocytes also displayed variation, and for this reason, on average, there was not a significant change in this marker postrace compared with prerace. Although the mechanisms through which exercise (especially eccentric and aerobic exercise) affects CAT activity still remain unknown, many studies have indicated that CAT activity did not change after exercise (38,50,78), which is in agreement with the finding of our study.

Another important question worth considering is the protective role of GSH against the oxidation of lipids and proteins. The study's hypothesis tested whether high GSH levels prerace prevent the oxidation of cellular proteins and lipids in athletes who underwent such a demanding competition. This is a crucial issue as a large majority of the athletes who partake in competitions and especially exhaustive running events could potentially use this information to reform their diet and supplementation regimens. However, in reviewing the individual responses, it seems that high GSH levels were not enough to achieve a protection from the oxidation of lipids and proteins after an exhaustive race. Namely, athletes with low GSH values prerace had reduced CARB and TBARS values postrace and vice versa. For example, individual No. 6 with GSH value prerace of 6.275 $\mu mol \cdot g^{-1}$ Hb exhibited increased TBARS and CARB levels postrace in contrast to No. 12 for whom the GSH value was 2.894 $\mu mol \cdot g^{-1}$ Hb prerace and exhibited a decrease in TBARS and CARB levels postrace. Therefore, it appears that the protection from oxidation of lipids and proteins has to do with multiple mechanisms in each organism and not only with GSH levels prerace. Of course, since the sample size was small, it should be taken caution regarding the above inference.

In conclusion, this is the first study in which the redox status of athletes who underwent an ultramarathon mountain race were tested beyond the immediate postrace time period and up to 3 days after the completion of the event on adaptations made by the body to protect against oxidative stress. A portion of our results suggest that oxidative stress was increased and present for up to 72 hours postrace compared with their respective prerace values. In addition, in all athletes, GSH levels postrace were considerably lower than prerace, apart from a slight increase in 3 samples at 72 hours (percentage change width was from −39.66 to −9.6% at 24 hours, from −54.39 to −9.5% and from −48.38 to 12.3% at 72 hours, respectively), suggesting that this antioxidant molecule was primarily consumed for counteracting ultramarathon mountain race–induced oxidative stress. However, the

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results from the individual analysis showed that protection from oxidation of lipids and proteins did not depend only on the GSH levels perace. Moreover, there were great variations in oxidative stress levels between different individuals. Importantly, there was even one individual (i.e., no. 1) in whom there was reductive stress instead of oxidative stress postrace. Other studies have also shown that eccentric exercise cannot only induce oxidative stress but also negligible stress or even reductive stress in a considerable number of people (39,66).

A possible explanation for this great variation is the high complexity of the regulation of redox homeostasis in human, since many genetic, physiological, biochemical, or dietary factors may affect the final outcome of oxidant stimuli (29,56,61). For example, among the genes whose variability seems to affect the response to oxidative stress are those encoding for superoxide dismutase, glutathione S-transferase, glutathione peroxidase, thioredoxin reductase, xanthine dehydrogenase, and genes involved in mitochondrial activity (13). In addition, the observed variations could be attributed to the altered integration of redox systems because of the conducted exercise. Integration refers to the characteristic of many biochemical systems, which display a correlation and interdependency in many levels (e.g., functional, structural, developmental, or evolutionary) (11,12). Moreover, another important consideration is that redox biomarkers are chemical substances, whose biological effects, multiple functional roles, actions, and metabolic pathways are still obscure (8,9,31). According to the literature, their levels may greatly vary in the general population and most of them exhibit a complex interdependency (3,12,69,74).

Practical Applications

The findings of this study could be useful for the understanding of oxidative stress induced by a demanding exercise such as an ultramarathon mountain race. This understanding of the oxidative stress induction would help the endeavor to improve immediate recovery process and consequently health status and performance of the athletes participating in such athletic events, by altering nutrition and/or certain antioxidant supplements. Thus, the present results suggested that ultramarathon mountain race–induced oxidative stress was sustained for up to 3 days postrace. The most effective markers for short-term monitoring of ultramarathon mountain race–induced oxidative stress were the novel ORP markers, sORP and cORP, as well as GSH levels, since they exhibited higher consistency between different athletes than the other tested markers, which showed great variability. Moreover, the great variability in some oxidative stress markers between different athletes suggested that general recommendations based on these markers for recovering from ultramarathon mountain race–induced oxidative stress is not the appropriate approach. It would be preferable that each marker to be examined individually to make the appropriate interventions. Furthermore, the results showed that GSH was mainly used for counteracting ultramarathon mountain race–induced oxidative stress, since all athletes exhibited a considerable reduction in GSH levels postrace that was also independent from the GSH levels prerace. However, low GSH levels have been associated with increased risk for several pathological conditions (70). Thus, it is suggested the administration of supplements during ultramarathon mountain races for enhancing especially GSH levels. Interestingly, in our previous studies, we have found a grape extract that increased antioxidant capacity in human cells by enhancing especially the GSH system (21–23).

References

1. Aebi, H. Catalase in vitro. *Methods Enzymol* 105: 121–126, 1984.
2. Alessio, HM, Hagerman, AE, Fulkerson, BK, Ambrose, J, Rice, RE, and Wiley, RL. Generation of reactive oxygen species after exhaustive aerobic and isometric exercise. *Med Sci Sports Exerc* 32: 1576–1581, 2000.
3. Arguelles, S, Gomez, A, Machado, A, and Ayala, A. A preliminary analysis of within-subject variation in human serum oxidative stress parameters as a function of time. *Rejuvenation Res* 10: 621–636, 2007.
4. Bakonyi, T and Radak, Z. High altitude and free radicals. *J Sports Sci Med* 3: 64–69, 2004.
5. Beaton, LJ, Tarnopolsky, MA, and Phillips, SM. Contraction-induced muscle damage in humans following calcium channel blocker administration. *J Physiol* 544: 849–859, 2002.
6. Bloomer, RJ, Goldfarb, AH, Wideman, L, McKenzie, MJ, and Consitt, LA. Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. *J Strength Cond Res* 19: 276–285, 2005.
7. Briviba, K, Watzl, B, Nickel, K, Kulling, S, Bos, K, Haertel, S, Rechkemmer, G, and Bub, A. A half-marathon and a marathon run induce oxidative DNA damage, reduce antioxidant capacity to protect DNA against damage and modify immune function in hobby runners. *Redox Rep* 10: 325–331, 2005.
8. Bruschi, M, Candiano, G, Santucci, L, and Ghiggeri, GM. Oxidized albumin. The long way of a protein of uncertain function. *Biochim Biophys Acta* 1830: 5473–5479, 2013.
9. Carocho, M and Ferreira, ICFR. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food Chem Toxicol* 51: 15–25, 2013.
10. Chiang, J, Shen, Y-C, Wang, Y-H, Hou, Y-C, Chen, C-C, Liao, J-F, Yu, M-C, Jual, C-W, and Liou, K-T. Honokiol protects rats against eccentric exercise-induced skeletal muscle damage by inhibiting NF-kappaB induced oxidative stress and inflammation. *Eur J Pharmacol* 610: 119–127, 2009.
11. Costantini, D, Monaghan, P, and Metcalfe, NB. Biochemical integration of blood redox state in captive zebra finches (Taeniopygia guttata). *J Exp Biol* 214: 1148–1152, 2011.
12. Costantini, D, Monaghan, P, and Metcalfe, NB. Loss of integration is associated with reduced resistance to oxidative stress. *J Exp Biol* 216: 2213–2220, 2013.
13. Dato, S, Crocco, P, D'Aquila, P, de Rango, F, Bellizzi, D, Rose, G, and Passarino, G. Exploring the role of genetic variability and lifestyle in oxidative stress response for healthy aging and longevity. *Int J Mol Sci* 14: 16443–16472, 2013.
14. Davies, KJ, Quintanilha, AT, Brooks, GA, and Packer, L. Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun* 107: 1198–1205, 1982.
15. Degache, F, Morin, J-B, Oehen, L, et al. Running Mechanics During the World's Most Challenging Mountain Ultramarathon. *Int J Sports Physiol Perform* 11: 608–614, 2016.

Copyright © National Strength and Conditioning Association Unauthorized reproduction of this article is prohibited.

16. DiLorenzo, FM, Drager, CJ, and Rankin, JW. Docosahexaenoic acid affects markers of inflammation and muscle damage after eccentric exercise. *J Strength Cond Res* 28: 2768–2774, 2014.

17. Elokda, A, DiFrancisco-Donoghue, J, Lamberg, EM, and Werner, WG. Effects of exercise induced oxidative stress on glutathione levels in Parkinson's disease on and off medication. *J Neurol* 257: 1648–1653, 2010.

18. Fatouros, IG, Jamurtas, AZ, Villiotou, V, Pouliopoulou, S, Fotinakis, P, Taxildaris, K, and Deliconstantinos, G. Oxidative stress responses in older men during endurance training and detraining. *Med Sci Sports Exerc* 36: 2065–2072, 2004.

19. Gambelunghe, C, Rossi, R, Micheletti, A, Mariucci, G, and Rufini, S. Physical exercise intensity can be related to plasma glutathione levels. *J Physiol Biochem* 57: 9–14, 2001.

20. Ghosh, J and Myers, CE. Inhibition of arachidonate 5-lipoxygenase triggers massive apoptosis in human prostate cancer cells. *Proc Natl Acad Sci U S A* 95: 13182–13187, 1998.

21. Goutzourelas, N, Stagos, D, Demertzis, N, Mavridou, P, Karterolioti, H, Georgadakis, S, Kerasioti, E, Aligiannis, N, Skaltsounis, LA, Statiri, A, Tsioutsiouliti, A, Tsatsakis, AM, Hayes, AW, and Kouretas, D. Effects of polyphenolic grape extract on the oxidative status of muscle and endothelial cells. *Hum Exp Toxicol* 33: 1099–1112, 2014.

22. Goutzourelas, N, Stagos, D, Housmekeridou, A, Karapouliou, C, Kerasioti, E, Aligiannis, N, Skaltsounis, LA, Spandidos, D, Tsatsakis, AM, and Kouretas, D. Grape pomace extract exerts antioxidant effects through an increase in GCS levels and GST activity in muscle and endothelial cells. *Int J Mol Med*, 2015.

23. Goutzourelas, N, Stagos, D, Spanidis, Y, Liosi, M, Apostolou, A, Priftis, A, Haroutounian, S, Spandidos, D, Tsatsakis, AM, and Kouretas, D. Polyphenolic composition of grape stem extracts affects antioxidant activity in endothelial and muscle cells. *Mol Med Rep* 12: 5846–5856, 2015.

24. Halliwell, B. The wanderings of a free radical. *Free Radic Biol Med* 46: 531–542, 2009.

25. Inayama, T, Kumagai, Y, Sakane, M, Saito, M, and Matsuda, M. Plasma protein-bound sulfhydryl group oxidation in humans following a full marathon race. *Life Sci* 59: 573–578, 1996.

26. Janaszewska, A and Bartosz, G. Assay of total antioxidant capacity: Comparison of four methods as applied to human blood plasma. *Scand J Clin Lab Invest* 62: 231–236, 2002.

27. Ji, LL, Gomez-Cabrera, M-C, and Vina, J. Exercise and hormesis: Activation of cellular antioxidant signaling pathway. *Ann N Y Acad Sci* 1067: 425–435, 2006.

28. Kabasakalis, A, Kyparos, A, Tsalis, G, Loupos, D, Pavlidou, A, and Kouretas, D. Blood oxidative stress markers after ultramarathon swimming. *J Strength Cond Res* 25: 805–811, 2011.

29. Kant, AK and Graubard, BI. Ethnic and socioeconomic differences in variability in nutritional biomarkers. *Am J Clin Nutr* 87: 1464–1471, 2008.

30. Kanter, MM, Lesmes, GR, Kaminsky, LA, La Ham-Saeger, J, and Nequin, ND. Serum creatine kinase and lactate dehydrogenase changes following an eighty kilometer race. Relationship to lipid peroxidation. *Eur J Appl Physiol Occup Physiol* 57: 60–63, 1988.

31. Kar, S and Kavdia, M. Endothelial NO and O(2). (-) production rates differentially regulate oxidative, nitroxidative, and nitrosative stress in the microcirculation. *Free Radic Biol Med* 63: 161–174, 2013.

32. Keles, MS, Taysi, S, Sen, N, Aksoy, H, and Akcay, F. Effect of corticosteroid therapy on serum and CSF malondialdehyde and antioxidant proteins in multiple sclerosis. *Can J Neurol Sci* 28: 141–143, 2001.

33. Kerksick, C and Willoughby, D. The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. *J Int Soc Sports Nutr* 2: 38–44, 2005.

34. Klapcinska, B, Waskiewicz, Z, Chrapusta, SJ, Sadowska-Krepa, E, Czuba, M, and Langfort, J. Metabolic responses to a 48-h ultra-marathon run in middle-aged male amateur runners. *Eur J Appl Physiol* 113: 2781–2793, 2013.

35. Kobayashi, Y, Nakatsuji, A, Aoi, W, Wada, S, Kuwahata, M, and Kido, Y. Intense exercise increases protein oxidation in spleen and liver of mice. *Nutr Metab Insights* 7: 1–6, 2014.

36. Liao, P, Zhou, J, Ji, LL, and Zhang, Y. Eccentric contraction induces inflammatory responses in rat skeletal muscle: Role of tumor necrosis factor-alpha. *Am J Physiol Regul Integr Comp Physiol* 298: R599–R607, 2010.

37. Malm, C, Lenkei, R, and Sjodin, B. Effects of eccentric exercise on the immune system in men. *J Appl Physiol (1985)* 86: 461–468, 1999.

38. Mangner, N, Linke, A, Oberbach, A, Kullnick, Y, Gielen, S, Sandri, M, Hoellriegel, R, Matsumoto, Y, Schuler, G, and Adams, V. Exercise training prevents TNF-alpha induced loss of force in the diaphragm of mice. *PLoS One* 8: e52274, 2013.

39. Margaritelis, NV, Kyparos, A, Paschalis, V, Theodorou, AA, Panayiotou, G, Zafeiridis, A, Dipla, K, Nikolaidis, MG, and Vrabas, IS. Reductive stress after exercise: The issue of redox individuality. *Redox Biol* 2: 520–528, 2014.

40. Margaritelis, NV, Veskoukis, AS, Paschalis, V, Vrabas, IS, Dipla, K, Zafeiridis, A, Kyparos, A, and Nikolaidis, MG. Blood reflects tissue oxidative stress: A systematic review. *Biomarkers* 20: 97–108, 2015.

41. McHugh, MP. Recent advances in the understanding of the repeated bout effect: The protective effect against muscle damage from a single bout of eccentric exercise. *Scand J Med Sci Sports* 13: 88–97, 2003.

42. Meeus, M, Nijs, J, Hermans, L, Goubert, D, and Calders, P. The role of mitochondrial dysfunctions due to oxidative and nitrosative stress in the chronic pain or chronic fatigue syndromes and fibromyalgia patients: Peripheral and central mechanisms as therapeutic targets? *Expert Opin Ther Targets* 17: 1081–1089, 2013.

43. Melikoglu, MA, Kaldirimci, M, Katkat, D, Sen, I, Kaplan, I, and Senel, K. The effect of regular long term training on antioxidant enzymatic activities. *J Sports Med Phys Fitness* 48: 388–390, 2008.

44. Mrakic-Sposta, S, Gussoni, M, Moretti, S, Pratali, L, Giardini, G, Tacchini, P, Dellanose, C, Tonacci, A, Mastorci, F, Borghini, A, Montorsi, M, and Vezzoli, A. Effects of mountain ultra-marathon running on ROS production and oxidative damage by micro-invasive analytic techniques. *PLoS One* 10: e0141780, 2015.

45. Mylonas, C and Kouretas, D. Lipid peroxidation and tissue damage. *In Vivo* 13: 295–309, 1999.

46. Nieman, DC, Dumke, CI, Henson, DA, McAnulty, SR, McAnulty, LS, Lind, RH, and Morrow, JD. Immune and oxidative changes during and following the western states endurance run. *Int J Sports Med* 24: 541–547, 2003.

47. Niess, AM, Hartmann, A, Grunert-Fuchs, M, Poch, B, and Speit, G. DNA damage after exhaustive treadmill running in trained and untrained men. *Int J Sports Med* 17: 397–403, 1996.

48. Nikolaidis, MG, Jamurtas, AZ, Paschalis, V, Kostaropoulos, IA, Kladi-Skandali, A, Balamitsi, V, Koutedakis, Y, and Kouretas, D. Exercise-induced oxidative stress in G6PD-deficient individuals. *Med Sci Sports Exerc* 38: 1443–1450, 2006.

49. Nikolaidis, MG, Kyparos, A, Hadziioannou, M, Panou, N, Samaras, L, Jamurtas, AZ, and Kouretas, D. Acute exercise markedly increases blood oxidative stress in boys and girls. *Appl Physiol Nutr Metab* 32: 197–205, 2007.

50. Oh-ishi, S, Kizaki, T, Nagasawa, J, Izawa, T, Komabayashi, T, Nagata, N, Suzuki, K, Taniguchi, N, and Ohno, H. Effects of endurance training on superoxide dismutase activity, content and mRNA expression in rat muscle. *Clin Exp Pharmacol Physiol* 24: 326–332, 1997.

51. Orient, A, Donkó, Á, Szabó, A, Leto, TL, and Geiszt, M. Novel sources of reactive oxygen species in the human body. *Nephrol Dial Transplant* 22: 1281–1288, 2007.

Copyright © National Strength and Conditioning Association Unauthorized reproduction of this article is prohibited.

52. Patsoukis, N, Zervoudakis, G, Panagopoulos, NT, Georgiou, CD, Angelatou, F, and Matsokis, NA. Thiol redox state (TRS) and oxidative stress in the mouse hippocampus after pentylenetetrazol-induced epileptic seizure. *Neurosci Lett* 357: 83–86, 2004.

53. Peake, J, Nosaka, K, and Suzuki, K. Characterization of inflammatory responses to eccentric exercise in humans. *Exerc Immunol Rev* 11: 64–85, 2005.

54. Phaneuf, S and Leeuwenburgh, C. Apoptosis and exercise. *Med Sci Sports Exerc* 33: 393–396, 2001.

55. Reddy, YN, Murthy, SV, Krishna, DR, and Prabhakar, MC. Role of free radicals and antioxidants in tuberculosis patients. *Indian J Tuberc* 51: 213–218, 2004.

56. Rankinen, T and Bouchard, C. Gene-physical activity interactions: Overview of human studies. *Obesity (Silver Spring)* 16(Suppl 3): S47–S50, 2008.

57. Reichhold, S, Neubauer, O, Bulmer, AC, Knasmüller, S, and Wagner, K-H. Endurance exercise and DNA stability: Is there a link to duration and intensity? *Mutat Res* 682: 28–38, 2009.

58. Rosa-Lima, FL, Lannes, L, Viana-Gomes, D, Pierucci, APTR, and Salerno, VP. Protein carbonyl levels correlate with performance in elite field hockey players. *Appl Physiol Nutr Metab* 40: 683–688, 2015.

59. Schneider, BSP and Tiidus, PM. Neutrophil infiltration in exercise-injured skeletal muscle: How do we resolve the controversy? *Sports Med* 37: 837–856, 2007.

60. Serravite, DH, Perry, A, Jacobs, KA, Adams, JA, Harriell, K, and Signorile, JF. Effect of whole-body periodic acceleration on exercise-induced muscle damage after eccentric exercise. *Int J Sports Physiol Perform* 9: 985–992, 2014.

61. Simoneau, JA and Bouchard, C. Human variation in skeletal muscle fiber-type proportion and enzyme activities. *Am J Physiol* 257: E567–E572, 1989.

62. Skenderi, KP, Tsironi, M, Lazaropoulou, C, Anastasiou, CA, Matalas, A-L, Kanavaki, I, Thalmann, M, Goussetis, E, Papassotiriou, I, and Chrousos, GP. Changes in free radical generation and antioxidant capacity during ultramarathon foot race. *Eur J Clin Invest* 38: 159–165, 2008.

63. Smith, JA. Exercise immunology and neutrophils. *Int J Sports Med* 18 (Suppl 1): S46–S55, 1997.

64. Spanidis, Y, Goutzourelas, N, Stagos, D, Kolyva, AS, Gogos, CA, Bar-Or, D, and Kouretas, D. Assessment of oxidative stress in septic and obese patients using markers of oxidation-reduction potential. *In Vivo* 29: 595–600, 2015.

65. Stagos, D, Goutzourelas, N, Bar-Or, D, Ntontou, A-M, Bella, E, Becker, AT, Statiri, A, Kafantaris, I, and Kouretas, D. Application of a new oxidation-reduction potential assessment method in strenuous exercise-induced oxidative stress. *Redox Rep* 20: 154–162, 2014.

66. Stagos, D, Goutzourelas, N, Ntontou, A-M, Kafantaris, I, Deli, CK, Poulios, A, Jamurtas, AZ, Bar-Or, D, and Kouretas, D. Assessment of eccentric exercise-induced oxidative stress using oxidation-reduction potential markers. *Oxid Med Cell Longev* 2015: 204615, 2015.

67. Steensberg, A, van Hall, G, Osada, T, Sacchetti, M, Saltin, B, and Klarlund Pedersen, B. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J Physiol* 529(Pt 1): 237–242, 2000.

68. Stupka, N, Tarnopolsky, MA, Yardley, NJ, and Phillips, SM. Cellular adaptation to repeated eccentric exercise-induced muscle damage. *J Appl Physiol (1985)* 91: 1669–1678, 2001.

69. Su, H, Gornitsky, M, Geng, G, Velly, AM, Chertkow, H, and Schipper, HM. Diurnal variations in salivary protein carbonyl levels in normal and cognitively impaired human subjects. *Age (Dordr)* 30: 1–9, 2008.

70. Townsend, DM, Tew, KD, and Tapiero, H. The importance of glutathione in human disease. *Biomed Pharmacother* 57: 145–155, 2003.

71. Tsai, K, Hsu, TG, Hsu, KM, Cheng, H, Liu, TY, Hsu, CF, and Kong, CW. Oxidative DNA damage in human peripheral leukocytes induced by massive aerobic exercise. *Free Radic Biol Med* 31: 1465–1472, 2001.

72. Turner, JE, Bennett, SJ, Bosch, JA, Griffiths, HR, and Aldred, S. Ultra-endurance exercise: Unanswered questions in redox biology and immunology. *Biochem Soc Trans* 42: 989–995, 2014.

73. Turner, JE, Hodges, NJ, Bosch, JA, and Aldred, S. Prolonged depletion of antioxidant capacity after ultraendurance exercise. *Med Sci Sports Exerc* 43: 1770–1776, 2011.

74. Valencia, E, Marin, A, and Hardy, G. Circadian rhythmicity of whole-blood glutathione in healthy subjects. *Nutrition* 17: 731–733, 2001.

75. Valko, M, Leibfritz, D, Moncol, J, Cronin, MTD, Mazur, M, and Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 39: 44–84, 2007.

76. Veskoukis, AS, Nikolaidis, MG, Kyparos, A, Kokkinos, D, Nepka, C, Barbanis, S, and Kouretas, D. Effects of xanthine oxidase inhibition on oxidative stress and swimming performance in rats. *Appl Physiol Nutr Metab* 33: 1140–1154, 2008.

77. Wiecek, M, Maciejczyk, M, Szymura, J, and Szygula, Z. Changes in oxidative stress and acid-base balance in men and women following maximal-intensity physical exercise. *Physiol Res* 64: 93–102, 2015.

78. Wiggs, MP. Can endurance exercise preconditioning prevention disuse muscle atrophy? *Front Physiol* 6, 2015.

79. Wilhelm, M, Zueger, T, De Marchi, S, Rimoldi, SF, Brugger, N, Steiner, R, Stettler, C, Nuoffer, J-M, Seiler, C, and Ith, M. Inflammation and atrial remodeling after a mountain marathon. *Scand J Med Sci Sports* 24: 519–525, 2014.

80. Zembron-Lacny, A, Naczk, M, Gajewski, M, Ostapiuk-Karolczuk, J, Dziewiecka, H, Kasperska, A, and Szyszka, K. Changes of muscle-derived cytokines in relation to thiol redox status and reactive oxygen and nitrogen species. *Physiol Res* 59: 945–951, 2010.

81. Zembron-Lacny, A, Slowinska-Lisowska, M, and Ziemba, A. Integration of the thiol redox status with cytokine response to physical training in professional basketball players. *Physiol Res* 59: 239–245, 2010.

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