Article

Oxidation of Human Serum Albumin Exhibits Inter-Individual Variability after an Ultra-Marathon Mountain Race

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Abstract: The aim of this study was to examine the oxidation of human serum albumin (HSA) caused by oxidative stress after an exhaustive exercise such as ultra-marathon race. Thus, blood samples from 12 adult runners who underwent a 103 km mountain ultra-marathon race were collected pre- and 24, 48 and 72 h post race. HSA was partially purified using affinity chromatography and then was subjected to Western blot analysis for disulfide dimers determination, indicating oxidation. The results were correlated with those from a previous study in which the same samples were analyzed using different oxidative stress markers and a good correlation with protein carbonyls (PC) at all time points was observed. Moreover there was a significant correlation with static oxidation-reduction potential (sORP) at 24 h, and a negative correlation with capacity oxidation-reduction potential (cORP) at 24 and 48 h. In addition, an individual analysis of albumin dimers exhibited great inter-individual differences. This inter-individual variability in the oxidation of HSA may suggest different interventions (e.g. through diet) in order to confront the effects on athletes' organism after a strenuous exercise. In conclusion, this study supported the importance of the assessment of albumin dimers as a predictive marker for exercise-induced oxidative stress.

Keywords: albumin, oxidative stress, exercise, ultra-marathon mountain race, protein oxidation

1. Introduction

Nowadays, a lot of studies have well established the association between physical exercise and the increase in the production of free radicals [1,2]. Free radicals are products of normal metabolism and include mainly reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet-}$), hydroxyl radical (OH•), and peroxyl radical (RO_2•), and reactive nitrogen species (RNS) such as nitric oxide (NO) and the peroxynitrite radical (ONOO•) [3]. Excessive production of ROS may affect several cell functions such as the regulation of signaling pathways and be involved in gene expression and apoptosis [4]. ROS generation is affected by endogenous sources such as mitochondrial respiratory chain, inflammation and cytochrome P450 activity [5] or exogenous sources like smoking, air pollution and UV light [6]. However, ROS generation during exercise and especially aerobic exercise is believed to be caused by the increased uptake of oxygen from the active peripheral skeletal muscle tissues [2]. The excessive ROS production may lead to a pathological condition called oxidative stress. For the determination of oxidative stress levels after exercise, a number of oxidative stress markers are assessed such as the levels and activity of antioxidant enzymes and molecules, oxidative DNA damage, lipid peroxidation and protein oxidation [7–9]

Regarding protein oxidation, it is usually assessed by measuring protein carbonyl levels (PC) in plasma. The most abundant protein in plasma (about 50% of total protein) is human serum albumin (HSA) [10]. HSA is a multifunctional, non-glycosylated globular protein composed of 585 amino acids with a molecular weight of 66 kDa and is mainly synthesized in the liver [11]. The structure of the protein contains a center made up of hydrophobic radicals used as a binding site for ligands, while the outer part is composed of hydrophilic ligands. More specifically, albumin

binds to and transfers several ligands such as bilirubin, hormones, metal ions and xenobiotics [12].

In addition, HSA possesses a free thiol group in Cys34, and thus it may function as an extracellular antioxidant by scavenging ROS [13,14]. Davies and co-workers have published a series of studies explaining in detail the relationship between oxidative damage and increased proteolytic susceptibility of bovine serum albumin (BSA) [15–18]. Particularly, albumin residues contain cysteine and methionine sulfhydryl groups reacting with peroxides leading to thiol oxidation [19,20]. It is believed that albumin acts as an antioxidant since albumin's Cys34, a cysteine that represents about 80% of total thiol content in plasma, scavenges ROS [21]. However, in oxidative stress conditions, albumin is oxidized and Cys34 forms a disulfide with low molecular weight thiols like cysteine. Thus, the oxidation caused by free radicals may affect the molecule's conformation and structure [22]. Actually albumin dimers have been reported as products of peroxidation caused by free radicals, and consequently they could be used as a marker of oxidative stress [23]. Moreover, since many studies have reported association between the oxidation of albumin and exercise [14,22,24], the determination of albumin dimer levels may be a good indicator of oxidative stress in athletes.

Therefore, the present study focused on the determination of the levels of albumin dimmers in plasma of runners participating in an exhaustive mountain marathon race, 'Olympus Mythical Trail 2015'. This mountain marathon race covers a distance of 103 km in the mountain of Olympus in Northern Greece. It is considered to be one of the most demanding routes worldwide, since it includes a 7,200 m elevation gain and a highest altitude of 2,906 m, while 40 km of the route take place at an altitude higher that 2,000 m.

Moreover, in one of our previous studies, protein oxidation in plasma of these runners was determined spectrophotometrically using PC assay in order to assess the redox status from 24 to 72 hours post-race [25]. Thus, it was also examined how the levels of HSA oxidation were correlated with PC levels of total plasma protein as well as with other oxidative stress markers. These correlations would help to examine if HSA oxidation is a good marker for the assessment of oxidative stress after exercise.

2. Materials and Methods

Subjects

Twelve (12) adult male runners aged 41.1 ± 3.2 years voluntarily participated in the present study (height 1.78 ± 0.02 m; weight 72.9 ± 2.0 kg). The subjects were informed not to receive any anti-inflammatory medicines or nutritional supplement and they were all familiar with mountain running.

The participants visited the Litohoro Health Center, located close to the starting point, 8 h before the race in order to complete a health and activity questionnaire and their anthropometric parameters were taken. Moreover, a written informed consent to participate in the study was provided by all the participants just before the blood collection. Body mass was measured to the nearest 0.5 kg (Beam balance 710, Seca, UK) with the subjects lightly dressed and barefoot. Standing height was measured to the nearest 0.5 cm (Stadiometer 208, Seca).

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the University of Thessaly (Project identification code: 1074, February 10th 2016).

Description of the race

The volunteers participated at one of the most extreme mountain ultra-marathons worldwide called ''Olympus Mythical Trail'' (OMT), at July 4-5th 2015 in Olympus Mountain in Northern Greece. The peculiarity and difficulty of the race lies on the fact that it is a 103 km ''loop'' type route having a total ascent (positive height difference) of 7,200 m (more than two times the altitude of Olympus Mountain) while about 40 km of it passed above 2,000 m altitude. The starting and ending points are placed at Litohoro town in Greece. The route consists mostly of paths (95%) and dirt (5%) and is divided into 18 checkpoints. The maximum time allowed for race completion was 28 h.

Subject's performance

After the completion of the race, 8 out of 12 participants achieved to finish the race, while 2 of them gave up at the 70th km and the other 2 at the 60th km (Table 1). The mean running time of the athletes was 19.57 ± 1.09 h.

Table 1. Depiction of each athlete's performance. For athletes who did not finish (DNF), the km in which they quit is also displayed.

Individual	Result
1	Finished
2	Finished
3	Finished
4	Finished
5	Finished
6	Finished
7	DNF (70th km)
8	DNF (60th km)
9	Finished
10	DNF (70th km)
11	Finished
12	DNF (60th km)

Blood collection and processing

The blood samples (10 ml) were drawn from a forearm vein with subjects in seated position at four different time points; 8 h before the competition (pre-race sample) and 24, 48 and 72 h post race. The samples were stored in ethylenediamine acid (EDTA) tubes and centrifuged at 1370 x g for 10 minutes at 4 °C to divide erythrocytes from the plasma. The plasma lysates were then stored at -80 °C prior to biochemical analysis.

Albumin determination assay

Albumin was determined spectrophotometrically at 628 nm, based on the formation of a coloured complex with bromocresol green reagent (BCG) solution in a 0.075 M succinate buffer (pH = 4.20)[26].

Partial purification of albumin

For sample preparation, 1 volume of plasma was diluted in 50 volumes of a 0.1 M HEPES buffer (pH 7.0), containing 1 mM EDTA (Buffer A). The column was equilibrated by 10 ml of Buffer A using an AKTA prime protein purification system (AKTA purifier UPC 10, GE Healthcare Bio-Sciences AB, Uppsala, Sweden), and then the diluted sample was applied to a Blue Sepharose column (1 ml) (HiTrap Blue HP, GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The column was washed with 10 ml of Buffer A, and the purified HSA was eluted and selected in a test tube using a 10 mL solution of 0,15 M KCl containing Buffer A (Buffer B).

Western blot analysis of albumin and albumin dimers

Protein concentration in plasma after the purification of the samples was measured using the Bradford reagent (Sigma-Aldrich Corp., St. Louis, MO). The calculation of the albumin concentration was made by using an albumin standard curve. Then, albumin monomers and dimers were determined in the purified plasma

samples with Western blot analysis by using a non-reducing SDS loading buffer. A non-reducing SDS loading buffer was used, as non-reducing conditions allow the visualization of any disulfide-linked dimers [27]. Specifically, non-reducing buffers do not contain beta-mercaptoethanol (2-ME) or dithiothreitol (DTT), which can reduce disulphide bridges in proteins. In order to perform immunoblot analysis, the purified sample was diluted until the final concentration of 1 μg of albumin was achieved. Afterwards, an aliquot containing the diluted purified sample and a 2x non-reducing loading buffer was prepared, heated in boiling water for 3 minutes and separated by SDS-PAGE, using a polyacrylamide gel 8% (w/v). After 1 h of electrophoresis at 150 V, proteins were transferred onto polyvinylidenedifluoride (PVDF) membranes (Biorad Laboratories, Hercules, CA) and blocked overnight with 5% non-fat milk in TBST (13 mmol/L Tris, 150 mmol/L NaCl (pH 7.5) solution, containing also 0.2% Tween-20 (TBSTMS).

Then, the membranes were incubated in a shaker for 1 h at room temperature with a goat anti-human albumin antibody diluted 1:5000 in TBSTMS. After extensive washes in TBST (5 times for 5 min) the blots were incubated for 30 min with anti-goat IgG secondary antibody (1:3000 dilution). The membranes washed in TBST (3 times for 15 min) and the labeled protein bands were visualized by enhanced chemiluminescence (Biorad Laboratories, Hercules, CA) and subsequent exposure to XAR 5 film (Fujifilm Corporation, Tokyo, Japan). The protein bands were quantified by using Alpha View quantification software (Alpha Innotech, San Leandro, CA). Every sample was analyzed in triplicate.

Statistical analysis

The statistical analysis was based on one-way ANOVA followed by Dunnett's test for multiple pairwise comparisons. The statistical significance level was set at p <

0.05.

Correlations between oxidized albumin and the other oxidative stress markers were examined by Spearman's correlation analysis. The level of significance was also set at $p < 0.05$. For all statistical analyses, SPSS version 20.0 (SPSS Inc., Chicago, Illinois, USA) was used. Data are presented as mean $\pm$ SEM.

3. Results

3.1. Western blot analysis for albumin dimers

Western blot analysis was used for the assessment of albumin monomers and dimers. Monomer bands were displayed at ~55 kDa, while dimers formation appeared at about ~110 kDa (Figure 1). The lower molecular weight of the dimer bands compared to the theoretical one has been observed previously [23].
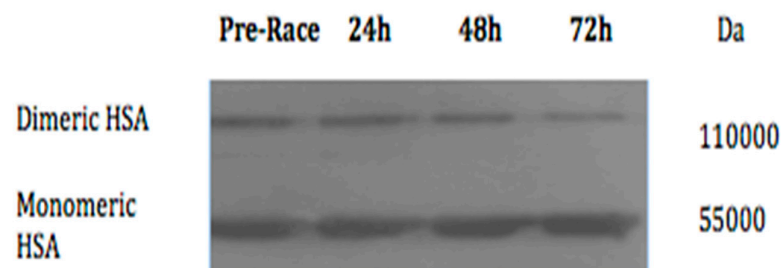

Figure 1. Representative picture for the detection of monomeric and dimeric serum albumin (HSA) using Western blot analysis. Specifically, it is shown the HSA from individual no. 2 at all time points (pre-race and 24, 48 and 72 h post-race). Albumin was obtained by Blue sepharose column chromatography and subjected to SDS–PAGE 8% (w/v) gel, under non-reducing conditions prior to immunoblotting analysis.

In each sample, the percentage ratio of dimers to monomers was quantified and considered as marker of HSA oxidation. The percentage change of oxidized HSA (i.e. the ratio of dimers to monomers) at 24, 48 and 72 h post race compared to pre race is shown in Figure 2. HSA oxidation was lower at all time points compared to pre race although not statistically significant (Figure 2).
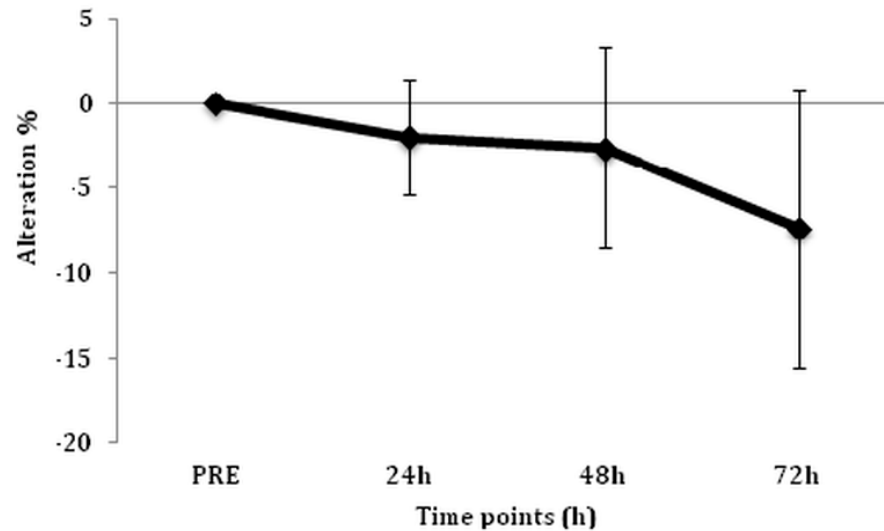
Figure 2. Percentage alteration of oxidized HSA (i.e. dimers/monomers ratio) and PC levels in the plasma samples from the athletes participating in the mountain marathon race at 24, 48 and 72h post race compared with pre race samples. The values indicate mean ± standard error of the mean.

Similarly, the percentage ratio of dimers to the total amount of HSA [i.e. dimers / (dimers + monomers)] was also quantified in order to obtain a clearer view regarding the changes of the protein after the race (Table 2).

Table 2. Percentage ratio of dimer HSA to total HSA of athletes at all time points.

	Oxidized /Total HSA levels (%)			
Time point (h) Individual	PRE	24	48	72
1	36.59	34.12	30.19	27.42
2	39.06	34.27	37.31	44.01
3	45.37	44.84	38.53	29.22
4	28.96	37.98	30.38	37.12
5	36.43	31.74	28.09	22.88
6	36.58	36.04	43.06	34.82
7	38.38	35.12	33.47	39.18
8	21.95	22.92	25.51	24.46
9	25.44	24.68	27.93	15.42
10	39.51	41.73	40.26	39.90
11	30.11	32.19	36.51	37.55
12	35.11	33.60	32.74	35.95
Mean	34.95±2.02	34.60±1.89	34.17±1.36	32.83±2.53

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3.2. Correlation with other oxidative stress markers as measured in the same samples in a previous study

In a previous study, we have assessed in the same samples the percentage changes at 24, 48 and 72 h post race of the oxidative stress markers PC, thiobarbituric acid reactive species (TBARS), glutathione levels (GSH), total antioxidant capacity (TAC), catalase activity (CAT), static oxidation-reduction potential (sORP) and capacity oxidation-reduction potential (cORP) compared to pre race [25]. In order to find out if the changes of HSA oxidation are associated with any other marker, a correlation analysis was carried out between the percentage changes of HSA at time points post race and the percentage changes of the other oxidative stress biomarkers (Table 3). The results showed that there was a significant high correlation between HSA oxidation and PC at all three time points post race (Table 3). There were also significant medium correlations between HSA oxidation and sORP at 24 h post race, as well as with cORP at 24 and 48 h post race (Table 3).

Table 3. Correlation analysis between percentage changes of HSA oxidation at 24, 48 and 72 h post race compared to pre race and the corresponding percentage changes of PC, TBARS, GSH, sORP and cORP oxidative stress markers.

	Time point (h)	PC	TBARS	GSH	TAC	CAT	sORP	cORP
HSA	24	0.769[*]	-0.329	0.448	-0.098	-0.147	0.58[*]	-0.601[*]
	48	0.867[**]	0.336	0.063	-0.329	-0.231	0.448	-0.657[*]
	72	0.860[**]	-0.091	-0.140	-0.007	-0.524	-0.105	0.056

[*]Significantly different correlation (p<0.05). [**]Significantly different correlation (p<0.01).

3.3. Individual analysis of HSA oxidation and correlation with PC

As in our previous work, we have shown that there were great differences in the percentage changes post race of oxidative stress markers including PC between different individuals [25] it was suggested that some markers should be examined individually in order to apply the appropriate interventions. Thus, the changes of

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HSA oxidation post race were also examined individually (Figures 3 and 4). Moreover, since HSA exhibited high correlation with PC showing the oxidation of total protein in plasma, the individual changes of HSA post race were displayed along with the individual changes of PC (Figures 3 and 4). This individual analysis showed that in some athletes (no. 6, 8, 10 and 11) HSA was clearly oxidized post race compared to pre race (Figures 3 and 4). However, in other athletes (no. 1, 3, 5, 7 and 9) HSA oxidation post race was much lower than that pre race indicating not oxidative but reductive stress (Figures 3 and 4). In three athletes (no. 2, 4 and 12) HSA oxidation had no changes between pre and post exercise (Figures 3 and 4). The comparison between HSA oxidation and PC showed that in some athletes HSA oxidation followed the same trend (i.e. increase or decrease) as PC (individuals no. 2, 3, 7, 8, 10, 11). However in one athlete (individual no.1), HSA seemed to be the main target of protein oxidation from ROS, while in others (individuals no 5, 4, 6, 9, 12). HSA was more protected from ROS compared with the total protein (Figures 3 and 4).
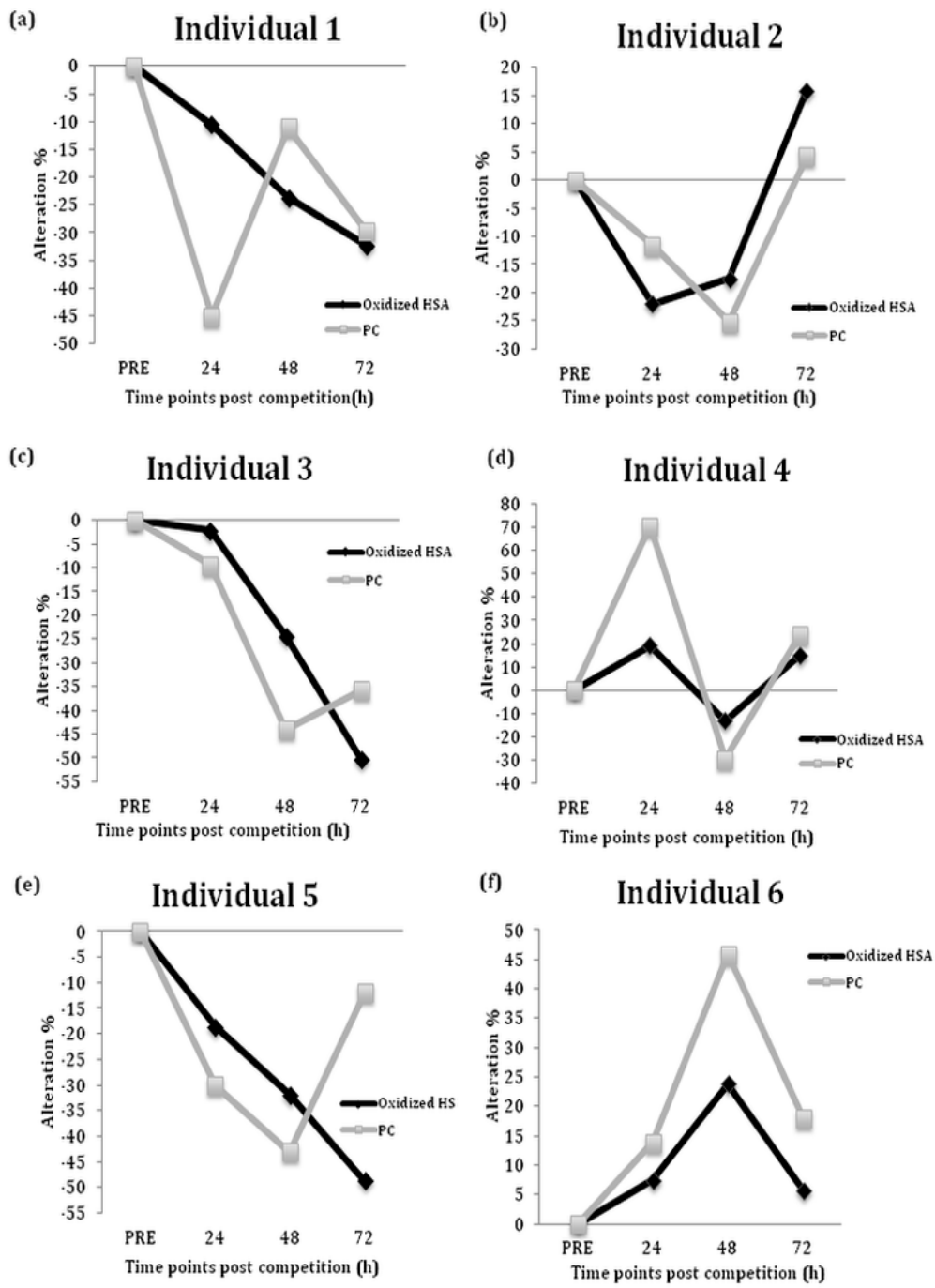
Figure 3. Percentage changes of HSA oxidation (i.e. dimers/monomers ratio), and PC levels of individuals 1-6 at 24, 48 and 72 hours post race time points, compared with pre-race.
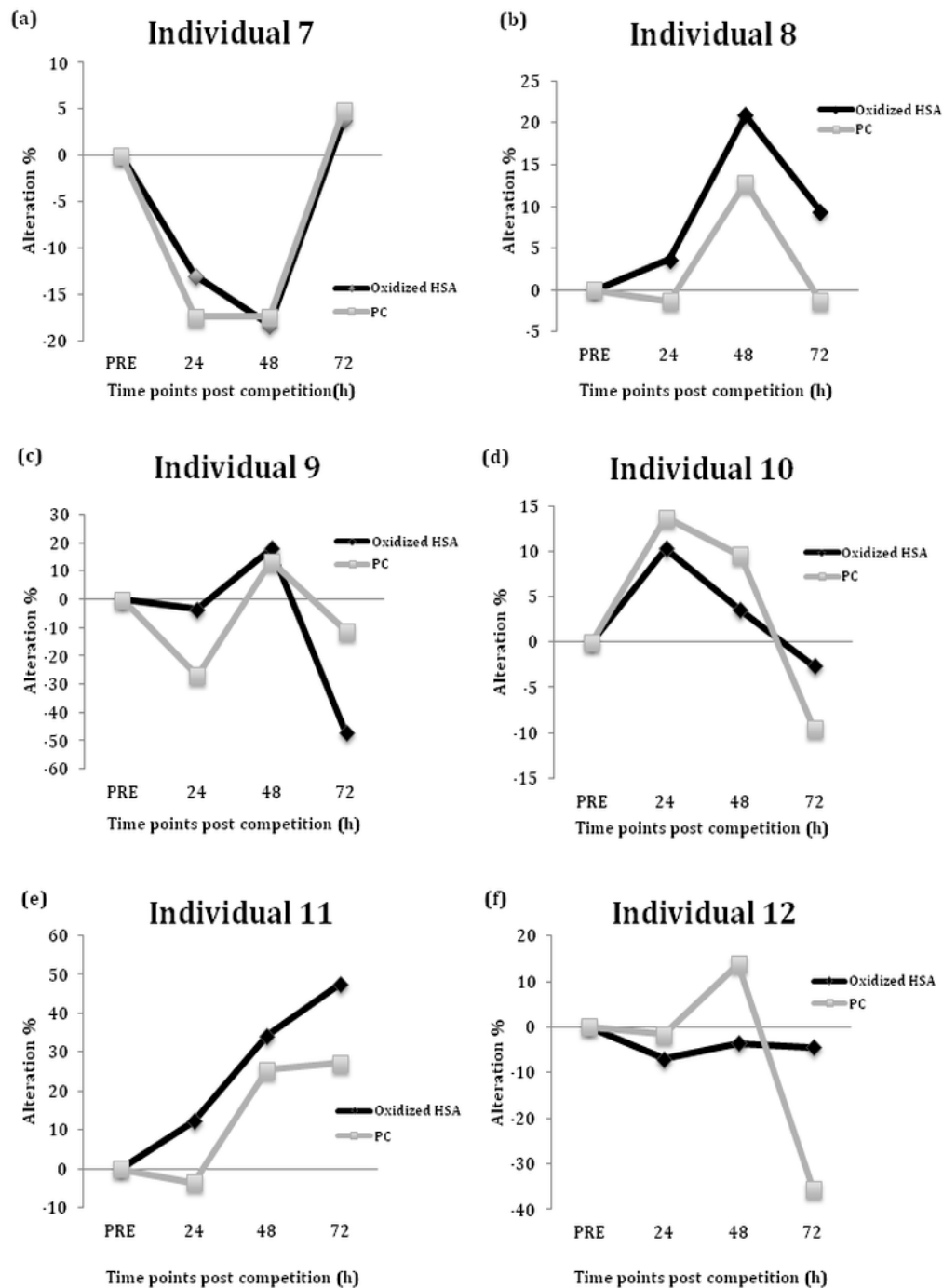
Figure 4. Percentage changes of HSA oxidation (i.e. dimers/monomers ratio), and PC levels of individuals 7-12 at 24, 48 and 72 hours post race time points, compared with pre-race.

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4. Discussion

In the present study, the changes of HSA oxidation were examined after a strenuous exercise such as a mountain-marathon race. Specifically, blood samples were collected from twelve experienced male mountain-marathon runners participated in a 103 km mountain marathon race, 'Olympus Mythical Trail'. Blood samples were collected from athletes at four different time points, pre race and 24, 48 and 72 h post race, in order to assess the alterations in their redox status by quantifying the HSA dimer formation, that is, the HSA oxidation.

HSA is the most abundant protein in plasma, as it makes up about 55% of the total serum protein content [28]. HSA's function is based on non-specific binding sites, which allow it to transfer a variety of molecules throughout the circulatory system. Specifically, it binds water, cations (e.g. Ca^{2+}, Na^+, K^+ etc.), fatty acids, hormones, pharmaceuticals and vitamins. Albumin's main function is the regulation of the colloidal osmotic pressure of blood [29]. Thus, many of the enzymatic activities of HSA are connected with the binding of metabolic products, which affects the related metabolic pathways [30] .

HSA contains a total of 35 cysteine residues from which 34 are involved in intramolecular disulfide bonds and only cysteine 34 (Cys34) remains free [31]. It is estimated that about 70% of the total free thiol content in plasma exists in HSA Cys34 [32]. This pool of thiol compounds in plasma gives rise to thiol exchange reactions leading to a number of disulfide bonds, and thus to formation of dimers acting as antioxidants by scavenging hydroxyl or other radicals through the reduced sulfhydryl group [33]. The dimerization site proved to be the Cys34 by forming a disulfide bridge between two albumin molecules [34]. According to Ogasawara et al., [23], the formed dimers as a result to ROS exposure can be used as oxidative stress marker. As

shown by us and others, formation of HSA dimers was also displayed after exhaustive exercise-induced oxidative stress [14,35]. It is noteworthy that exercise-induced increase in ROS activates adaptive responses through signaling pathways regulated by thiol status, including reduced Cys34 of HSA [22,36–38]. Additionally, changes in thiol redox status induce the expression of nuclear factor-kB (NF-kB) and activator protein-1 (AP-1) leading to an increase in the levels of the cytokines IL-6 and TNF-a [36,39]. Both of these cytokines not only affect muscle regeneration, but also the development of tolerance after ROS-induced muscle damage. In general, oxidation of a protein and more specifically HSA after ROS exposure, can lead to a loss of its structural and catalytic function [40]. Oxidation and thus dimerization of HSA impedes its activity, as the dimer is more rigid, rendering substrate binding less favorable [41]. Specifically, oxidation of HSA has been observed to decrease both the ligand binding property of site II and the esterase-like activity of HSA most probably due to conformational changes in subdomain IIIA [41]. In addition, HSA exhibited an intristic enolase activity towards dihydrotestosterone that was reduced upon dimerization [30]. Moreover, when oxidized proteins are accumulated in the cells, then degradation systems are activated [42]. Therefore, excessive HSA oxidation should be prevented. The significant interplay between HSA and oxidative stress necessitates the investigation of HSA oxidation, especially in athletes undergoing demanding exercise.

The present results showed that on average HSA oxidation was not increased at any time point post race compared to that one pre race. Although there was not oxidation of thiols of HSA post race compared to pre race, thiol groups of glutathione of the same samples were oxidized as shown previously [25]. Therefore, HSA seems to be more protected against oxidation than GSH, since the latter may be involved

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more in ROS scavenging. Our results was in agreement with one of our previous studies on the same samples, which has shown that there was not any difference in the oxidation of total protein in plasma between pre and post race [25]. However, other studies have shown increase in HSA oxidation after exercise [14,22,35]. As we have reported previously, this discrepancy between different studies regarding protein oxidation may be explained by the inter-individual variation of protein oxidation after strenuous exercise [25]. Namely, in 9 athletes oxidation of total protein was increased post exercise compared to pre exercise, while the other 3 athletes exhibited decrease post exercise [25]. Likewise, HSA oxidation was increased in 4 athletes, decreased in 5 athletes and had no change in 3 athletes post race compared to pre race.

The samples used in the present study were also analyzed in one of our previous studies by measuring other oxidative stress markers such as PC, TBARS, TAC, GSH, CAT, sORP and cORP [25]. Thus, HSA oxidation was assessed by quantifying dimers, so as to compare its levels with the other performed assays and make conclusions about its usefulness as a biomarker for strenuous exercise-induced oxidative stress. The results showed significantly high correlation between HSA oxidation and PC (i.e oxidation of total protein) at all time points post race. The aforementioned correlation was expected, since the increased concentration of PC after exercise has been suggested to be mainly derived from the oxidation of HSA making up about 55% of total serum protein as well as of other major proteins [43,44]. In addition, there was a significant positive correlation between HSA oxidation and sORP at 24 hours post race, and negative correlation with cORP at 48 and 72 hours post race respectively. These two novel markers have been used previously in several of our studies for assessing exercise-induced oxidative stress [7–9,45]. The above correlations were meaningful, since sORP increased values

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correspond to higher oxidative stress levels, as it represents the integrated balance of oxidants and reductants, while cORP is a measure of antioxidant reserve available in the body's system.

As mentioned above, HSA oxidation exhibited a great variation between different athletes. Moreover, HSA oxidation had a high correlation with PC, that is, with oxidation of total plasma protein. Thus, each athlete was examined separately in order to compare the changes of HSA oxidation and PC levels, and so to identify the extent at which HSA oxidation affects the total protein oxidation after exhaustive exercise. Several studies have demonstrated that albumin as well as fibrinogen are the main protein targets of oxidative stress in plasma [43,44,46,47]. Moreover, since HSA represents about the half of the total protein content in plasma, a similar trend of PC and HSA oxidation levels in each individual was expected post race. However, our results indicated that protein oxidation seems not to be affected so much by HSA oxidation. Specifically, the results showed that similar oxidation levels between PC and HSA at all time points post race were displayed in only 5 out of 12 runners (individuals no. 2, 7, 8, 10 and 11). Thus, in these individuals, it seems that HSA affects mainly the total protein oxidation post race. However, the rest 7 athletes exhibited great differences in the changes of oxidation between PC and HSA at one or more time points post race. It was remarkable, that in 6 athletes HSA was more protected from oxidation than the other serum proteins. These results supported hypothesis of Madian et al., who suggested that despite HSA's abundance in plasma, it is not so vulnerable to oxidation as other proteins like fibrinogen [47,48]. To sum up, all these findings suggested that the measurement of only PC is not sufficient to make conclusions about HSA oxidation, and thus it is needed to be examined separately after exercise.

Moreover, in a previous study, we have shown that in some of these athletes instead of protein oxidation post race, there was protein reduction (individuals no. 1, 3, 5, 7 and 9) [25]. Similarly, albumin in these athletes was shown reduction and not oxidation post race. The manifestation of reductive stress instead of oxidative one, especially after eccentric exercise, has been reported by us as well as by others [7,49]. This intriguing effect can be explained by 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 [50–52].

The changes of the ratio of dimer to total HSA post exercise compared to pre exercise showed the same trend with the ratio of dimer to monomer HSA, that is, in each athlete both ratios either decreased or increased post exercise. Like ratio of dimer to monomer HSA, the ratio of dimer to total HSA exhibited great variability, from 15.42 to 45.37%, between different individuals at all time points post exercise. This variability in HSA oxidation may indicate differences in its functionality, that is, individuals with higher HSA oxidation levels are likely to have lower HSA activity and vice versa. As mentioned above, lower HSA functionality may affect its binding capacity for several ligands, and thus there may be need for a dietary intervention in order to improve the athletes' redox status especially after strenuous exercise.

In conclusion, the present results supported the notion that the assessment of HSA dimmers, that is, HSA oxidation may be used as a marker of oxidative stress after exhaustive exercise. Especially the correlation between HSA oxidation and other oxidative stress markers such as PC, sORP and cORP supported this inference. In general, thiol levels have been suggested as a marker of oxidative stress [29]. However, the assessment of oxidative stress using low molecular weight thiols is difficult, because they are susceptible to oxidative damage and their measurement,

especially in blood, is not easy. Thus, the measurement of stable oxidized thiol groups, such as albumin dimmers, is more practical [23]. Moreover, the fact that in some athletes the changes of HSA oxidation post exercise did not follow the changes of PC suggested the need for assessing both of these markers in order to reach a more confident conclusion about protein oxidation in plasma and make the appropriate interventions. Finally, this study showed for the first time that in some athletes HSA was reduced instead of oxidized post exercise, highlighted the need for investigating further the individual impact on HSA oxidation and generally in redox status. The understanding of the inter-individual variability of HSA oxidation could be useful for applying the appropriate interventions through nutrition and supplementation to athletes participating in demanding exercise such as mountain marathon race. This individual approach would help athletes to better improve immediate recovery process and consequently health status and performance

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Conflicts of Interest: The authors declare no conflict of interest

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