Single bouts of exercise affect albumin redox state and carbonyl groups on plasma protein of trained men in a workload-dependent manner

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Lamprecht M, Greilberger JF, Schwaberger G, Hofmann P, Oettl K. Single bouts of exercise affect albumin redox state and carbonyl groups on plasma protein of trained men in a workload-dependent manner. J Appl Physiol 104: 1611–1617, 2008. First published April 17, 2008; doi:10.1152/japplphysiol.01325.2007.—The purpose of this study was to investigate the effect of single bouts of exercise at three different intensities on the redox state of human serum albumin (HSA) and on carbonyl groups on protein (CP) concentrations in plasma. Trained men (n = 44, maximal oxygen consumption (V0₂max): 55 ± 5 ml·kg⁻¹·min⁻¹, nonsmokers, 34 ± 5 years of age] from a homogenous population, volunteers from a police special forces unit, were randomly assigned to perform on a cycle ergometer either at 70% (n = 14), 75% (n = 14), or 80% (n = 16) of V0₂max for 40 min. Blood was collected before exercise, immediately after the exercise test (IE), and 30 min after each test (30M) and 30 h after each test (30H). The reduced fraction of HSA, human mercaptalbumin (HMA), decreased at all three exercise intensities IE and 30M, returning to preexercise values by 30H (P < 0.05). HMA was primarily oxidized to its reversible fraction human nonmercaptalbumin 1 (HNA1). CP concentrations increased at 75% of V0₂max IE and 30M with a tendency (P < 0.1) and at 80% V0₂max IE and 30M significantly, returning to preexercise concentrations by 30H (P < 0.01). These results indicate that the HSA redox system in plasma is activated after a single bout of cycle ergometer exercise at 70% V0₂max and 40 min duration. The extent of the HSA modification increased with exercise intensity. Oxidative protein damage, as indicated by CP, was only significantly increased at 80% V0₂max intensity in this homogenous cohort of trained men.

NUMEROUS OXIDATIVE stress-related exercise studies and reviews report that physical exercise can result in increased generation of reactive oxygen and nitrogen species (RONS) (1, 12, 33). It is thought that production of these RONS during endurance exercise is due to the delivery of increased amounts of oxygen to active peripheral skeletal muscle tissue (33). RONS are eliminated by the antioxidant system that comprises endogenous antioxidant enzyme systems (SOD or glutathione peroxidase (GPx)) and defined exogenous substances like vitamins or polyphenols (27, 34). If antioxidant defenses are overwhelmed by the generation of reactive oxygen and nitrogen compounds, oxidative processes dominate and result in a condition referred to as “oxidative stress.” Consequently, lipids, proteins, and DNA can be damaged (30). In athletes, oxidative stress can result in muscular oxidative damage (15), muscle soreness (32), loss of skeletal muscle force production (19), or subsequent inflammation (11).

Under exercise conditions, the amount of molecule oxidation in body fluids and tissues is determined, e.g., by intensity and duration of exercise, the kind of muscle contraction, or the availability of exogenous dietary antioxidants (5, 12, 35, 39, 40). Additionally, the efficiency of the endogenous antioxidant enzyme system influences the outcome of exercise-induced free radical damage, and the quality of this system seems to depend in part on the individual level of fitness (27, 30, 34, 36). Under the viewpoint of these determinants, a variety of different oxidative stress markers are reported by literature, but there is a lack of standardization of these variables, e.g., with regard to intensity, duration, type of exercise, or antioxidant intake.

The main protein in extracellular fluids, human serum albumin (HSA), acts as transport and redox system. Its redox state might be affected by physical exercise (17). Therefore, in this study, we describe a new approach to investigate oxidative processes in exercise by estimating the redox state of HSA concerning cysteine-34. In the fully reduced form of HSA, cysteine-34 contains a free thiol group (human mercaptalbumin, HMA). In a mild oxidized form, cysteine-34 forms a disulfide with a small-molecular-weight thiol like cysteine (human nonmercaptalbumin 1, HNA1). Finally, cysteine-34 may be further oxidized to the sulfenic or sulfonic acid form (human nonmercaptalbumin 2, HNA2) (14). HMA and HNA1 are reversibly interconvertible, but oxidation to HNA2 is an irreversible step (8). To the best of our knowledge, no study has been published concerning the possible influence of different defined exercise intensities on the redox state of HSA.

Carbonyl groups on protein (CP) are generated with protein oxidation and are often used as systemic markers of oxidative stress (6, 13, 23). Several studies demonstrated higher concentrations of CP after strenuous exercise (3, 5, 25, 28). The higher concentrations of CP after exercise are thought to be derived, in part, by oxidized albumin (21, 23).

The aim of this study was to determine the values of the oxidation markers HMA, HNA1, HNA2, and CP before and after single bouts of cycle ergometer exercise at three different and defined intensities. We hypothesized that the extent of exertion might influence the redox state of HSA and the concentrations of CP in plasma of trained men. Additionally, we detected antioxidant enzyme activities of SOD and GPx to estimate possible differences between the groups, especially after exercise. To approach an adjustment of antioxidant intake

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between the subjects before exercise, the probationers were instructed by a dietician, 7-day food records were analyzed, and a standardized breakfast was consumed before the exercise tests.

MATERIALS AND METHODS

Subjects. All subjects provided written informed consent before participating in this investigation. This study was conducted according to the guidelines of the Declaration of Helsinki for research on human subjects 1989 and approved by the Ethics Committee at the Medical University of Graz. The cohort consisted of 44 healthy men, all nonsmokers, of police special anti-terrorism forces ("Cobra") who participated in this study. All subjects completed a medical history, dietary analysis, physical activity questionnaire, and body fat determination within 6 wk (recruitment period) before the beginning of the investigation to determine eligibility. Exclusion criteria included use of tobacco products, chronic or excessive alcohol consumption, recent surgery or illness, use of pharmaceuticals, drugs, or antioxidant-containing dietary supplements at least 4 wk before the exercise tests. All subjects trained aerobically at least 3 days per week for a minimum of a year before participation and had a minimum level of aerobic fitness as assessed with maximal testing (>50 m·kg⁻¹·min⁻¹). Body fat content and distribution were estimated by a computerized optical device (Lipometer; Möller Messtechnik, Graz, Austria), as described by Möller, et al. (24).

Study design and time schedule. This was a randomized, double-blind study: the laboratory team responsible for the biochemical analysis received no information about group differences from the exercise laboratory team. The probands had to perform their strenuous endurance tests on the prescribed intensity as instructed by the exercise physiologist. After the recruitment period, the study was conducted over an 8-wk experimental period, beginning with a 4-wk wash-out phase from any supplement. After the 4-wk wash-out period and after an overnight fast, the subjects came to the laboratory and blood samples were drawn for determination of a clinical blood chemistry panel. A few days afterward, they completed an incremental exercise test on a bicycle ergometer, including electrocardiography for determination of maximal oxygen consumption (V Õ₂ max) and to control heart function. Randomization to the three different exercise intensities followed. At least 1 wk but not longer than 2 wk after the maximal testing, the endurance tests at 70, 75, and 80% of V Õ₂ max were performed on the same ergometer, at standardized room temperature (22°C), humidity (60%), and after a standardized breakfast.

Incremental exercise tests. All subjects performed an incremental cycle ergometer exercise test (model no. ERG 9000; Schiller, Baar, Switzerland) at 80 revolution/min. After a 3-min rest phase sitting inactive on the ergometer, work rate started at 40 W for 3 min and was increased 20 W every minute until voluntary exhaustion. This allowed subjects to reach exhaustion within 15–18 min. A standard electrocardiogram was recorded during the entire test, which was supervised by a physician.

Respiratory gas exchange. Respiratory gas exchange variables were measured throughout all tests by using a breath-by-breath mode with data being stored in 10-s intervals. During all tests, subjects breathed through a facemask. Oxygen uptake (V Õ₂), carbon dioxide output (V Õ₂), minute ventilation (V̇), breathing rate, and tidal volume (V T) were continuously obtained by means of a portable open-air spirometry system (MetaMax I; Cortex Biophysics, Leipzig, Germany). The analyzers were calibrated with gases of known concentration before the tests according to the manufacturer’s guidelines. Heart rate was monitored throughout the tests using a commercially available heart rate monitor (Polar Vantage NV; Polar Electro, Kempele, Finland). Data were measured and stored in 5-s intervals.

Determination of ventilatory and lactate threshold. The second turn points for ventilation (V T 2) and blood lactate concentration (LT₃₇) were determined in all groups by means of linear regression break point analysis within defined regions (16). Target loads were set to guarantee that intensities could be maintained for at least 40 min in all subjects.

70, 75, and 80% V Õ₂ max exercise tests. Subjects were randomly assigned to three exercise tests: at 70% (n = 14), 75% (n = 14), and 80% (n = 16) of individual V Õ₂ max with 80 revolution/min on the same cycle ergometer for 40 min or break-off due to exhaustion. All exercise tests were carried out 3 h after a standardized breakfast/meal. Similar to the maximal test, subjects completed a rest phase on the ergometer for 3 min. Thereafter, exercise started at 40 W with 80 revolution/min for 3 min, and work rate was increased by 20 W every minute until the workload at 70, 75, or 80% of V Õ₂ max was reached, as calculated from the incremental V Õ₂ max test. V Õ₂ intensity was controlled throughout all tests to maintain the target percentage of V Õ₂ max intensity. Data from subjects who performed more than 2.4% apart from the target intensity were excluded from statistical analyses. Gas exchange variables were monitored continuously throughout the exercise test as described above. After 20 min on defined intensity, facemask was removed briefly to consume 250 ml of water. Test stopped after 40 min on defined exercise intensity or after reaching exhaustion. Exhaustion was defined with inability to maintain performance (W) and 80 revolution/min at the specific percentage of V Õ₂ max.

Dietary assessment and stabilization. In the 6-wk recruitment period, all subjects were instructed by a dietician to maintain their habitual diet during the experimental period and to consume 7-day food record for nutrient intake assessment. Subjects subsequently received copies of their 7-day diet records and were instructed to replicate the diet before the exercise tests. Breakfast 3 h before each exercise test was standardized for the entire cohort to limit nutrient variation due to self-selection on the morning scheduled for blood draws. Diet records were analyzed for total calories, proteins, carbohydrates, fat, cholesterol, fiber, water, alcohol, and several vitamins and minerals using “opti diet” software (GOEmbH, Lindern, Germany).

Physical activity during testing. Each subject was instructed not to perform physical training 3 days before the exercise test and within 30 h after the test (30H), until after the last blood sample was drawn.

Blood collection and sample preparation. We conducted four blood collections per subject at each 40-min exercise test: before exercise (BE) at rest, immediately after each exercise test (IE), 30 min after the tests (30M), and 30H. Blood was collected to determine CP, HMA, HNA1, HNA2, and, additionally, antioxidant enzyme activities of SOD and GPx. Collection of capillary blood (600 μl) used EDTA-coated vials (Sarstedt, Graz, Austria). After centrifugation at 3,000 g for 10 min, plasma was removed and samples were frozen at −70°C until analysis of CP, HMA, HNA1, and HNA2. Albumin was washed three times with isotonic solution and then lyzed with Millipore H₂O. After centrifugation at 3,000 g for 10 min, the supernatant was frozen at −70°C for analysis of Hb, SOD, and GPx.

Analysis of HMA, HNA1, and HNA2. Albumin was fractionated by high-performance liquid chromatography to give three peaks according to cysteine-34 redox state, either in the free thiol form (HMA), as a mixed disulfide (HNA1), or in a higher oxidation state (HNA2), as previously described (18). Quantification was on the basis of the peak heights of the fractions compared with standards. Data are expressed as the percent serum albumin as HMA, HNA1, and HNA2. The analytical interassay coefficient of variance for these parameters was <3.5%.

Analysis of CP. Measurement of CP was done with a sensitive chemiluminescent immunosay (Lumistar, BMG, Offenburg, Germany) after derivatization with dinitrophenylhydrazine (DNPH) and the usage of anti-DNPH-antibody (Sigma, St. Louis, MO) on 96 microtitration plates (Nunc, Roskilde, Denmark) as described elsewhere (22). Assessment of plasma protein concentration used the
bichinonic assay (BCA; Pierce, Rockford, IL). The analytical interassay coefficient of variance for CP was <3.2%.

Analysis of SOD and GPx activity. Assessment of SOD activity used erythrocyte lysate with xanthine oxidase in the start reagent, as previously described (29), with results expressed in U/mg Hb. Determination of GPx activity from erythrocyte lysate was performed indirectly, by a coupled reaction with glutathione reductase, utilized the ZeptoMetrix (Buffalo, NY, USA) assay kit adapted to 96-well-plates, with results expressed in U/g Hb. The analytical interassay coefficient of variance for these parameters was <6.4%.

As the redox state of HSA is expressed as the fraction of HMA, HNA1, and HNA2, respectively, and CP, GPx, and SOD values are related to the total plasma protein content or Hb, possible dehydration by exercise has no influence on these parameters.

Blood chemistry panel. Standard blood chemistry values were determined by using 5 ml EDTA plasma from peripheral venous blood. Analysis used routine methods and the clinical chemistry analyzer “Eurolyser” (Dia Team, Diagnostica und Arzneimittel Großhandel, Linz, Austria). Assessment of Hb and iron concentrations used the Advia clinical analyzer (Bayer, Leverkusen, Germany).

Statistical analyses. All statistical analyses were performed using SPSS for windows software, version 12.0. Data are presented as means ± SD. Statistical significance was set at P < 0.05. Baseline characteristics, performance data, and nutrient and clinical chemistry data were compared between the three groups using one-way ANOVA. The data obtained for CP, HMA, HNA1, HNA2, SOD, and GPx were analyzed using repeated measures ANOVA to estimate the effects of the defined exercise intensities on these variables. Additionally, baseline values of CP, HMA, HNA1, HNA2, SOD, and GPx were compared by one-way ANOVA to estimate group differences at rest BE. We used Pearson regression analysis and correlation coefficient to evaluate bivariate relationships.

RESULTS

Characteristics of the study population, VO2max, and nutrition. The three groups did not differ in age, height, weight, total body fat, lean body mass, VO2max, and maximum workload (P > 0.05, Table 1). The analysis of the 7-day food records for daily kJ and macro- and micronutrient intake found no differences between the groups for any measured nutrient variable (P > 0.05, data not shown). Standardized breakfast 3 h before each exercise test provided about 4,222 kJ, 32–34 g protein, 144–150 g carbohydrate, and 28–30 g fat (Table 2). Clinical blood chemistry variables showed no differences between the groups (P > 0.05, Table 1).

Threshold data. VT2 and LTP2 were found at 79 ± 3.5% and 78.1 ± 3.6% of VO2max, respectively, and were not significantly different between groups (P > 0.1, Table 1). Exercise in the 70% VO2max group was significantly below VT2 and/or LTP2 exercise intensity (~10%), whereas exercises in the 75 and 80% VO2max groups were slightly below (75% group) or closely above (80% group) VT2 and/or LTP2.

Endurance exercise data. At 70% of individual VO2max, the group performed in mean at 230 ± 15 W for 39.8 ± 1.4 min and at 70 ± 2.4% of individual VO2max. The 75% VO2max group performed at 240 ± 10 W for 39.3 ± 2.1 min and at 75 ± 2.4% of individual VO2max. The 80% VO2max group performed at 250 ± 15 W for 37.6 ± 4.3 min and at 80 ± 2.4% of individual VO2max. No differences between the groups were noted for mean duration on specific percentage of VO2max (P > 0.05).

Values of HMA, HNA1, and HNA2. There were no differences between the three groups in resting HMA, HNA1, and HNA2 percentages BE (P > 0.1). Overall HMA percentages ranged from 63.1 to 73.2%. As shown in Fig. 1, there were significant effects to lower HMA percentages IE and 30M, returning to preexercise percentages at 30H in all three groups (P < 0.05). This effect was more pronounced the more the intensity of exercise increased: P = 0.041 at 70% VO2max, P = 0.037 at 75% VO2max, P < 0.001 at 80% of VO2max exercise.

Overall HNA1 percentages ranged from 22.1 to 33.5% and were increased toward a trend IE and 30M at 70 and 75% VO2max, returning to preexercise percentages at 30H in all three groups (P < 0.05). This effect was more pronounced the more the intensity of exercise increased: P = 0.011. The data are shown in Fig. 2. HNA2 percentages ranged from 1.6 to 3.1% in all and did not change at any intensity (P > 0.1, data not shown).

CP. There were no differences between the three groups in resting CP concentrations BE (P > 0.1). Overall CP conen-

Table 1. Characteristics and clinical chemistry data of 44 trained men

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference Range</th>
<th>70% VO2max (n=14)</th>
<th>75% VO2max (n=14)</th>
<th>80% VO2max (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>34.3 ± 5.1</td>
<td>33.8 ± 4.7</td>
<td>35.1 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>183.2 ± 6.4</td>
<td>180.8 ± 4.6</td>
<td>182.6 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>83.6 ± 8.1</td>
<td>79.8 ± 5.4</td>
<td>81.5 ± 6.3</td>
<td></td>
</tr>
<tr>
<td>Total body fat, %</td>
<td>12.9 ± 3.8</td>
<td>12.4 ± 2.2</td>
<td>11.9 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>72.8 ± 6.6</td>
<td>69.9 ± 4.7</td>
<td>71.8 ± 5.2</td>
<td></td>
</tr>
<tr>
<td>VO2max, ml·kg⁻¹·min⁻¹</td>
<td>58.4 ± 5.5</td>
<td>54.8 ± 4.3</td>
<td>56.4 ± 5.4</td>
<td></td>
</tr>
<tr>
<td>VT2; % of VO2max</td>
<td>79.0 ± 3.8</td>
<td>78.2 ± 3.2</td>
<td>79.7 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>LTP2; % of VO2max</td>
<td>78.1 ± 3.1</td>
<td>77.4 ± 2.9</td>
<td>78.8 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>Lactate during exercise, mmol/l</td>
<td>3.32 ± 0.08</td>
<td>3.67 ± 0.96</td>
<td>5.95 ± 2.68*</td>
<td></td>
</tr>
<tr>
<td>Maximum workload, W</td>
<td>340 ± 25</td>
<td>350 ± 15</td>
<td>345 ± 20</td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>(3.9–6.1)</td>
<td>4.8 ± 2.1</td>
<td>4.6 ± 1.2</td>
<td>4.9 ± 0.8</td>
</tr>
<tr>
<td>Hemoglobin, g/l</td>
<td>(136–172)</td>
<td>156 ± 28</td>
<td>158 ± 31</td>
<td>152 ± 26</td>
</tr>
<tr>
<td>Iron, µmol/l</td>
<td>(14–32)</td>
<td>19 ± 6</td>
<td>18 ± 7</td>
<td>21 ± 5</td>
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<tr>
<td>Cholesterol, mmol/l</td>
<td>(&lt;5.85)</td>
<td>4.45 ± 1.15</td>
<td>4.70 ± 1.00</td>
<td>4.56 ± 1.23</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>(&lt;1.80)</td>
<td>0.86 ± 0.48</td>
<td>0.94 ± 0.62</td>
<td>0.87 ± 0.36</td>
</tr>
<tr>
<td>Albumin, g/l</td>
<td>(40–60)</td>
<td>43 ± 15</td>
<td>44 ± 9</td>
<td>47 ± 12</td>
</tr>
<tr>
<td>C-reactive protein, mg/l</td>
<td>(0.5–30)</td>
<td>8 ± 19</td>
<td>9 ± 15</td>
<td>12 ± 16</td>
</tr>
<tr>
<td>Uric acid, µmol/l</td>
<td>(120–420)</td>
<td>210 ± 104</td>
<td>290 ± 82</td>
<td>243 ± 96</td>
</tr>
</tbody>
</table>

Values are means ± SD and did not differ between the groups (P > 0.05, ANOVA). *except for lactate concentrations during 80% of VO2max exercise (P < 0.05). Reference range for clinical chemistry parameters (Ref. 42). VO2max, maximal oxygen consumption; VT2, second turn point for ventilation; LTP2, second turn point for blood lactate concentration.
trations ranged from 0.21 to 0.59 nM/mg protein. As shown in Fig. 3, there was no significant effect on CP concentrations IE, 30M, or 30H at 70 and 75% of V˙O2max, although there was a tendency toward increased concentrations IE and 30M at 75% V˙O2max after 40 min of exercise (P < 0.1). Exercise at 80% of V˙O2max and 40 min significantly increased values IE and 30M, decreasing to preexercise concentrations at 30H (P < 0.01).

Correlation analyses. A trend to an inverse correlation was found when HMA values were compared with CP concentrations at 75% of V˙O2max (P = 0.088, r² = 0.831). This inverse correlation was significant at 80% V˙O2max: P = 0.020, r² = 0.961. Whereas HMA percentages decreased IE and 30M, CP concentrations increased at these time points. Both variables returned to baseline at 30H. A trend to a positive correlation was found when HNA1 values were compared with CP concentrations at 75% V˙O2max intensity (P = 0.067, r = 0.933). This correlation was significant at 80% V˙O2max exercise (P = 0.02, r = 0.998): both variables increased IE and 30M, returning to preexercise values after 30H.

SOD and GPx activity. There were no differences between the three groups in resting enzyme activities BE (P > 0.1).

Overall SOD activities ranged from 10.4 to 31.2 U/mg Hb, GPx activities from 126 to 262 U/g Hb. There were no effects on SOD activities at any exercise intensity (P > 0.1). This goes to GPx as well (data not shown).

DISCUSSION

In this study, we investigated the responses of protein oxidation markers in plasma and antioxidant enzymes in erythrocytes to three different cycle exercise intensities over 40 min. The data show that 1) the redox state of HSA was influenced...
by each exercise intensity in a dose-dependent manner: HMA percentage was reduced IE and 30M with recovery to near resting values by 30H postexercise. 2) Whereas HNA2 percentages did not change at any VO2max intensity, HMA was mainly oxidized to the reversible fraction HNA1 at IE and 30M and recovered to the reduced HMA fraction after 30H. 3) The data of this investigation indicate that significant protein damage occurs only at 80% VO2max exercise intensity with 40 min of duration, which was slightly above VT2/LTP2 threshold values. 4) At 80% VO2max cycle exercise intensity for 40 min, an inverse correlation between HMA percentages and CP concentrations was found from pre- to postexercise. Also a positive correlation between HNA1 and CP concentrations occurred at 80% VO2max intensity. 5) The antioxidant enzyme activities of SOD and GPx in erythrocytes were not influenced by any exercise intensity.

Oxidation of protein molecules can lead to a loss of catalytic or structural function in the affected protein (20). Proteolytic degradation is observed in several pathological states when oxidized proteins accumulate in cells, contributing to the progression of disease (4). For this reason, excessive protein oxidation should be avoided.

We hypothesized that the redox state of HSA as well as CP concentrations after strenuous exercise might be influenced in a workload- or intensity-dependent manner. Comparable studies have found higher plasma CP values after aerobic exercises performed at 70, 75, or 80% VO2max (5, 6, 7, 13, 28). In accordance with these studies, we chose our intensities at 70, 75 and 80% of VO2max to ensure a change at least in CP concentrations and to prove our hypothesis of intensity-dependent changes of HSA and CP. With 40 min of exercise duration at all three chosen intensities, we took advantage of the longest possible duration our subjects could perform at 80% of VO2max, a workload slightly above VT2/LTP2, to ensure supreme exertion at least with this protocol. We also observed a change in CP at 75% of VO2max IE and 30M with a trend, and at 80% VO2max the increase IE and 30M was significant. The baseline concentrations in our study were similar to those in the Bloomer (5) and Goldfarb (13) studies (~0.30 nM/mg protein), but we did not find this tremendous threefold increase. Furthermore, at 70% VO2max cycle exercise with similar duration, we could not observe an increase in CP concentrations as published in an actual study by Bloomer et al. (5). This apparent conflict could be due to the different testing protocols used to assess VO2 values, which can lead to different VO2max peak values. Shorter protocols (8 min) lead to higher values than longer protocols (16 min) as demonstrated recently by Yoon et al. (41). Our protocol duration of the incremental step test was longer (15–18 min). Therefore, our estimated VO2max peak, and, consequently, the VO2max percentages could have been lower for individual exertion than those of Bloomer’s (8–12 min). However, our study results confirm that oxidative protein damage occurs at least at 80% VO2max exercise for 40 min in trained men and also when we investigated protocols were applied.

Changes of the binding properties of albumin in response to oxidative modification are quite diverse and have been reviewed recently (26). The main fraction of serum albumin, HMA, contributes to the maintenance of a constant redox potential with its free thiol group, thus securing a certain redox buffer capacity in extracellular fluids (10). In addition, the distribution of HSA fractions can be used as a systemic redox marker, because albumin is the most abundant protein in plasma and responsible for the largest fraction of reactive sulphydryl (17). Therefore, HSA could become of further interest for exercise research to estimate the redox situation in plasma as other thiols like glutathione or lipoic acid already are (7, 21, 34). To the best of our knowledge, the redox state of HSA at defined exercise intensities and duration has not yet been reported.

Albumin makes up ~55% of total serum protein content, whereas 10 other abundant proteins account for more than 90% of all serum proteins (2). Therefore, some researchers postulate that the increased concentration of CP after exercise should be mainly derived from the oxidation of albumin and other major serum proteins (21, 23). Comparing HSA responses at the different exercise intensities to CP responses, we observed that albumin is oxidized at all exercise intensities, whereas CP concentrations start to increase at 75% of VO2max. This supports the hypothesis that the oxidation of protein thiols to mixed disulfides may be an early response to oxidative stress as postulated by other researchers (38). The HSA redox buffer system seems to act at the beginning of the radical scavenging chain in plasma, whereas CP concentrations represent the extent of overcharge of the plasma antioxidant systems.

Our P values demonstrate significant HMA oxidation already at 70 and 75% of VO2max (P = 0.041 and P = 0.037 respectively), but oxidation at 80% VO2max was significantly higher (P < 0.001), with a significant inverse correlation to the CP course at this intensity. These results also suggest that the HSA redox system is “consumed” first by generated free radicals, and the oxidation of HMA is more pronounced at exercise intensities of 80% VO2max compared with intensities of 70 and 75% VO2max. On the other hand, we are aware of the fact that these data do not allow us to estimate how much oxidized albumin contributes to the postexercise CP increase. For clarity, all abundant plasma proteins have to be analyzed and compared with CP concentrations.

Concerning recovery-to-baseline values, the data show that 30 h are sufficient to recover plasma proteins from oxidative modification as indicated by both HMA fraction and plasma CP concentration. In the case of HSA, the results confirm that this type of exercise only oxidized HMA to its reversible fraction HNA1 and did not provoke manifest damage to HSA as shown by unaffected HNA2 percentages at all exercise intensities.

The antioxidant enzyme activities of SOD and GPx in erythrocytes were monitored in this investigation. Changes of SOD and GPx activities postexercise or in recovery have been reported (31, 37). We failed with our exercise protocols to provoke changed SOD and GPx activities in these trained men. As postulated in some studies (9, 27), trained people might have adapted antioxidant enzyme systems. This is also suggested by our data showing high values of erythrocyte antioxidant enzyme activities in all groups. We believe that the main reason for the unaffected enzyme activities is due to the fitness level in this trained cohort.

In conclusion, the results of this study indicate that the HSA redox system in plasma acts at the beginning of the radical scavenging chain. The redox pool of HSA is more “consumed” with increasing exercise intensity. Plasma CP concentrations as indicator of oxidative protein damage increased significantly
only at intensities of 80% \( \dot{V}O_{2\text{max}} \) after 40 min of exercise on the cycle ergometer. Despite protein oxidation in plasma, erythrocyte antioxidant enzyme activity was unaffected.

The HMA response to exercise will require further investigation to explore the potential of this biomarker as a valuable redox sensor, e.g., to avoid protein damage induced by oxidative stress in exercise or to steer training load and recovery.

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