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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Running title: exercise intensity and protein oxidation
ABSTRACT

The purpose of this study was to investigate the effect of single bouts of exercise at 3 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, VO₂max: 55 ± 5 mL·kg⁻¹·min⁻¹, non-smokers, 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 VO₂max for 40 minutes. Blood was collected before exercise (BE), immediately after the exercise test (IE), 30 minutes (30M) and 30 hours (30H) after each test. The reduced fraction of HSA, human mercapt albumin (HMA), decreased at all 3 exercise intensities IE and 30M, returning to pre-exercise values by 30H (P < 0.05). HMA was primarily oxidized to its reversible fraction human nonmercapt albumin 1 (HNA1). CP concentrations increased at 75% of VO₂max IE and 30M with a tendency (P < 0.1) and at 80% VO₂max IE and 30M significantly, returning to pre-exercise 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% VO₂max and 40 minutes duration. The extent of the HSA modification increased with exercise intensity. Oxidative protein damage, as indicated by CP, was only significantly increased at 80% VO₂max intensity in this homogenous cohort of trained men.

Key Words: albumin modification, carbonylated protein, defined exertion
INTRODUCTION

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 compounds like the enzymes superoxide dismutase (SOD) or glutathione peroxidase (GPx) as well as exogenous nutritional 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 influenced 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 sulfinic 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 our best 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 carbonyl groups on protein (CP), before and after single bouts of cycle ergometer exercise, at 3 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 superoxide dismutase (SOD) and glutathione peroxidase (GPx) to estimate possible differences between the groups, especially after exercise. To approach an adjustment of antioxidant intake between the subjects before exercise, the probationers were instructed by a dietician, 7-d food records were analyzed and a standardized breakfast was consumed before the exercise tests.

MATERIALS AND METHODS

Subjects. All subjects provided written informed consent prior to 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 local Ethics Comitee. The cohort consisted of 44 healthy men, non smokers, 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 weeks (recruitment period) prior to 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 four weeks prior to the exercise tests. All subjects trained aerobically at least three days per week for a minimum of a year prior to participation and had a minimum level of aerobic fitness as assessed with maximal testing (>50 mL·kg⁻¹·min⁻¹). Body fat content and distribution was 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 probationers 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-week experimental period, beginning with a 4-week wash-out phase from any supplement. After the 4-week 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 afterwards, they completed an incremental exercise test on a bicycle ergometer, including electrocardiography for determination of VO₂max and to control heart function. Randomization to the 3 different exercise intensities followed. At least 1 week but not longer than 2 weeks after the maximal testing the endurance tests at 70%, 75% and 80% of VO₂ 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 (Schiller "ERG 900S" Ergometer, Switzerland) at 80 rpm. After a three minute rest phase sitting inactive on the ergometer, work rate started at 40 W for three minutes 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 (RGE). RGE variables were measured throughout all tests using a breath-by-breath mode with data being stored in 10s intervals. During all tests, subjects breathed through a facemask. Oxygen uptake (VO$_2$), carbon dioxide output (VCO$_2$), minute ventilation (V$_E$), breathing rate (BR) and tidal volume (V$_T$) were continuously obtained by means of a portable open-air spiroergometry system (MetaMax I, Cortex Biophysik, Leipzig, Germany). The analyzers were calibrated with gases of known concentration before the tests according to the manufacturer’s guidelines. Heart rate (HR) was monitored throughout the tests using a commercially available heart rate monitor (Polar Vantage NV, Polar Electro Finland). Data were measured and stored in five second intervals.

Determination of ventilatory and lactate threshold. The second turn points for ventilation (VT$_2$) and blood lactate concentration (LTP$_2$) 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 can be maintained for at least 40 minutes in all subjects.

70%, 75% and 80% VO$_2$max exercise tests. Subjects were randomly assigned to 3 exercise tests: at 70% (N = 14), 75% (N = 14) and 80% (N = 16) of individual VO$_2$max with 80 rpm on the same cycle ergometer for 40 min or break-off due to exhaustion. All exercise tests were carried out 3 hours after a standardized breakfast/meal. Similar to the maximal test, subjects completed a rest phase on the ergometer for three minutes. Thereafter, exercise started at 40 W with 80 rpm for three minutes and work rate was increased by 20 W every minute until the workload at 70%, 75% or 80% of VO$_2$max was reached, as calculated from the incremental VO$_2$max test. VO$_2$-intensity was controlled throughout all tests to maintain the target %VO$_2$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 minutes 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 rpm at specific percentage of VO$_2$max.

Dietary assessment and stabilization. In the 6-week recruitment period all subjects were instructed by a dietician for maintaining their habitual diet during the experimental period and to complete 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 prior to the exercise tests. Breakfast 3 hours 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, Linden, Germany).

Physical activity during testing. Each subject was instructed not to perform physical training 3 days prior to the exercise test and within 30 hours after the test, 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 minutes (30M) and 30 hours (30H) after the tests. Blood was collected to determine CP, HMA, HNA1, HNA2 and additionally, antioxidant enzyme activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx). Collection of capillary blood (600 µL) used EDTA coated vials (Sarstedt, Graz, Austria). After centrifugation at 3000 x g for 10 min plasma was removed and samples were frozen at -70°C until analysis of CP and HMA, HNA1 and HNA2. Erythrocytes were washed 3 times with isotonic solution and then lysed with
Millipore H$_2$O. After centrifugation at 3000 x g for 10 min, the supernatant was frozen at -70°C for analysis of hemoglobin (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 based on the peak heights of the fractions compared to standards. Data are expressed as the percent serum albumin as HMA, HNA1, and HNA2. The analytical inter-assay coefficient of variance for these parameters was < 3.5%.

**Analysis of CP.** Measurement of CP was done with a sensitive chemiluminescent immunoassay (Lumistar, BMG, Germany) after derivatization with di-nitrophenyl-hydrazine (DNPH) and the usage of anti-DNPH-antibody (Sigma, USA) on 96 microtitration plates (Nunc, Denmark) as described elsewhere (22). Assessment of plasma protein concentration used the bicinchoninic assay (BCA; Pierce, MD, USA). The analytical inter-assay 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 units/mg Hb. Determination of GPx activity from erythrocyte lysate was performed indirectly, by a coupled reaction with glutathione reductase, utilized the ZeptoMetrix Corporation (Buffalo, NY, USA) assay kit adapted to 96-well-plates, with results expressed in units/g Hb. The analytical inter-assay 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 GmbH,
Linz, Austria). Assessment of Hb and iron concentrations used the Advia clinical analyzer (Fa. Bayer, Leverkusen, Germany).

Statistical Analyses. All statistical analyses were performed using SPSS for windows software, version 12.0. Data are presented as mean ± SD. Statistical significance was set at P < 0.05. Baseline characteristics, performance data, nutrient and clinical chemistry data, were compared between the 3 groups using a one-way analysis of variance (ANOVA). The data obtained for CP, HMA, 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, 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 3 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-d food records for daily kJ, macro- and micronutrient intake found no differences between the groups for any measured nutrient variable (P > 0.05, data not shown). Standardized breakfast 3 hr before each exercise test provided about 4222 kJ, 32-34 g protein, 144-150 g carbohydrate, 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 Watt for 39.8 ± 1.4 min and at 70 ± 2.4% of individual VO₂max. The 75% VO₂max
group performed at 240 ± 10 Watt for 39.3 ± 2.1 min and at 75 ± 2.4% of individual VO₂max. The 80% VO₂max group performed at 250 ± 15 Watt 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 %VO₂max (P > 0.05).

Values of HMA, HNA1 and HNA2. There were no differences between the 3 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 pre exercise percentages at 30H in all 3 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 pre exercise percentages at 30H (P = 0.087 and P = 0.074 respectively). At 80% VO₂max this course was significant (P = 0.011). The data are shown in Figure 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).

Carbonyl groups on protein. There were no differences between the 3 groups in resting CP concentrations BE (P > 0.1). Overall CP concentrations ranged from 0.21 to 0.59 nM · mg protein⁻¹. As shown in Fig. 3, there was no significant effect on CP concentrations IE, 30M or 30H at 70% and 75% of VO₂max, although there was a tendency to increased concentrations IE and 30M at 75% VO₂max after 40 min exercise (P < 0.1). Exercise at 80% of VO₂max and 40 min significantly affected to increased values IE and 30M decreasing to pre-exercise concentrations at 30H (P < 0.01).

Correlation analyses. A trend to an inverse correlation was found when HMA values were compared to CP concentrations at 75% of VO₂max (P = 0.088, r² = 0.831). This inverse correlation was significant at 80% VO₂max: P = 0.020, r² = 0.961. While 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 to CP concentrations at 75% VO2max intensity (P = 0.067, R = 0.933). This correlation was significant at 80% VO2max exercise (P = 0.02, R = 0.998): both variables increased IE and 30M, returning to pre exercise values after 30H.

SOD and GPx activity. There were no differences between the 3 groups in resting enzyme activities BE (P > 0.1). Overall SOD activities ranged from 10.4 to 31.2 units · mg Hb⁻¹, GPx activities from 126 to 262 units · g Hb⁻¹. 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 3 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 30-hours post-exercise. 2) As 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 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 post exercise. 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 aerobic 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 proof our hypothesis of intensity dependent changes of HSA and CP. With 40 minutes of exercise duration at all 3 chosen intensities we took advantage of the longest possible duration our subjects could perform at 80% of VO2max, a work load 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⁻¹), but we did not find this tremendous 3-fold increase. Further, 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, also when our investigation 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 sulfhydryl
(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 our best knowledge the redox state of HSA at defined exercise intensities and duration has
not been reported yet.
Albumin makes up approximately 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 to intensities of 70% and 75% VO2max. On the other hand we are aware of that
these data do not allow to estimate how much oxidized albumin contributes to the post
exercise CP increase. For clarity, all abundant plasma proteins have to be analyzed and
compared to CP concentrations.
Concerning recovery to baseline values, the data show that 30 hours are sufficient to recover plasma proteins from oxidative modification as indicated by both, HMA fraction and plasma CP concentration. In case of HSA the results confirm that this types of exercise only oxidized HMA to it’s 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 post exercise 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% VO2max after 40 min 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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25. **Morillas-Ruiz JM, Villegas Garcia JA, Lopez FJ, Vidal-Guevara ML,** and **Zafrilla P.**


TABLE 1  Characteristics and clinical chemistry data of 44 trained men

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference range²</th>
<th>70% VO₂max (n = 14)</th>
<th>75% VO₂max (n = 14)</th>
<th>80% VO₂max (n = 16)</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>34.3 ± 5.1</td>
<td>33.8 ± 4.7</td>
<td>35.1 ± 4.6</td>
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<tr>
<td>Height, cm</td>
<td>183.2 ± 6.4</td>
<td>180.8 ± 4.6</td>
<td>182.6 ± 7.2</td>
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<tr>
<td>Weight, kg</td>
<td>83.6 ± 8.1</td>
<td>79.8 ± 5.4</td>
<td>81.5 ± 6.3</td>
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<tr>
<td>Total body fat, %</td>
<td>12.9 ± 3.8</td>
<td>12.4 ± 2.2</td>
<td>11.9 ± 3.3</td>
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<td>Lean body mass, kg</td>
<td>72.8 ± 6.6</td>
<td>69.9 ± 4.7</td>
<td>71.8 ± 5.2</td>
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<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>
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<td>VT2; % of VO₂max</td>
<td>79.0 ± 3.8</td>
<td>78.2 ± 3.2</td>
<td>79.7 ± 3.5</td>
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<td>LTP2; % of VO₂max</td>
<td>78.1 ± 3.1</td>
<td>77.4 ± 2.9</td>
<td>78.8 ± 4.0</td>
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<td>Lactate during exercise, mMol·L⁻¹</td>
<td>3.32 ± 0.81</td>
<td>3.67 ± 0.96</td>
<td>5.95 ± 2.68*</td>
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<td>Maximum workload, W</td>
<td>340 ± 25</td>
<td>350 ± 15</td>
<td>345 ± 20</td>
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<tr>
<td>Glucose, mmol·L⁻¹</td>
<td>(3.9-6.1)</td>
<td>4.8 ± 1.1</td>
<td>4.6 ± 1.2</td>
<td>4.9 ± 0.8</td>
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<td>Hemoglobin, g·L⁻¹</td>
<td>(136–172)</td>
<td>156 ± 28</td>
<td>158 ± 31</td>
<td>152 ± 26</td>
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<td>Iron, µmol·L⁻¹</td>
<td>(14 – 32)</td>
<td>19 ± 6</td>
<td>18 ± 7</td>
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<td>Cholesterol, mmol·L⁻¹</td>
<td>(&lt;5.85)</td>
<td>4.45 ± 1.15</td>
<td>4.70 ± 1.00</td>
<td>4.56 ± 1.23</td>
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<td>Triglycerides, mmol·L⁻¹</td>
<td>(&lt;1.80)</td>
<td>0.86 ± 0.48</td>
<td>0.94 ± 0.62</td>
<td>0.87 ± 0.36</td>
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<td>Albumin, g·L⁻¹</td>
<td>(40–60)</td>
<td>43 ± 15</td>
<td>44 ± 9</td>
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<td>C-reactive protein, mg·L⁻¹</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⁻¹</td>
<td>(120–420)</td>
<td>210 ± 104</td>
<td>290 ± 82</td>
<td>243 ± 96</td>
</tr>
</tbody>
</table>

¹Values 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)

²Reference range for clinical chemistry parameters (42)
TABLE 2
Composition of standardized breakfast 3 hr prior to strenuous endurance exercise

<table>
<thead>
<tr>
<th>Food</th>
<th>kJ</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Carbohydrates (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee with milk (low fat) or Tea with lemon and honey (10g)</td>
<td>180</td>
<td>0-2</td>
<td>0-2</td>
<td>4-10</td>
</tr>
<tr>
<td>3 slices wheat or rye bread</td>
<td>1390</td>
<td>8</td>
<td>1</td>
<td>75</td>
</tr>
<tr>
<td>Butter 20g</td>
<td>652</td>
<td>-</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Marmalade/jam 30g</td>
<td>343</td>
<td>-</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td>One slice low fat ham</td>
<td>331</td>
<td>6</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>One piece of cheese</td>
<td>490</td>
<td>16</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>250mL fruit juice</td>
<td>836</td>
<td>2</td>
<td>-</td>
<td>46</td>
</tr>
<tr>
<td>250mL water</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>4222</td>
<td>32-34</td>
<td>28-30</td>
<td>144-150</td>
</tr>
<tr>
<td>Meal energy %</td>
<td></td>
<td>13%</td>
<td>27%</td>
<td>60%</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

FIGURE 1 – Human mercapt albumin (HMA) percentages before exercise (BE), immediately post exercise (IE), 30 minutes post exercise (30M), and 30 hours post exercise (30H) for three different exercise intensities on a cycle ergometer and 40 minutes duration: at 70% of VO_2max peak, 75% and 80% of VO_2max peak. Values are means ± SD.
* Significant decrease IE and 30M, reaching BE concentrations at 30H post exercise. 70% VO_2max: n = 14, P = 0.043; 75% VO_2max: n = 14, P = 0.037; 80% VO_2max: n = 16, P < 0.001.

FIGURE 2 – Human nonmercaptalbumin 1 (HNA1) percentages: See note for Figure 1.
# At 70% and 75% VO_2max the values tended to increase post exercise, reaching BE concentrations at 30H post exercise (P < 0.1). Values are means ± SD.
* Significant increase IE and 30M, reaching BE values at 30H post exercise (P < 0.05).

FIGURE 3 – Carbonyl groups on protein (CP) concentrations. See note for Figure 1. Values are means ± SD.
# At 75% VO_2max concentrations tended to increase post exercise, reaching BE concentrations at 30H post exercise (P < 0.1).
* At 80% VO_2max significant increase to higher concentrations post exercise, reaching BE concentrations again at 30H post exercise (P < 0.01).
FIGURE 2

HNA1 (%) over Time

- 70% VO$_2$max
- 75% VO$_2$max
- 80% VO$_2$max

Time (BE, IE, 30M, 30H)