Moderate Exercise Blunts Oxidative Stress Induced by Normobaric Hypoxic Confinement

TADEJ DEBEVEC[1], VINCENT PIALOUX[2], IGOR B. MEKJAVIC[1], OLA EIKEN[3], PAULINE MURY[2], and GRÉGOIRE P. MILLET[4]

[1]Department of Automation, Biocybernetics and Robotics, "Jozef Stefan" Institute, Ljubljana, SLOVENIA; [2]Center of Research and Innovation on Sports, University Claude Bernard Lyon 1, Villeurbanne, FRANCE; [3]Department of Environmental Physiology, School of Technology and Health, Royal Institute of Technology, Stockholm, SWEDEN; and [4]Faculty of Biology and Medicine, ISSUL, Institute of Sport Sciences, University of Lausanne, Lausanne, SWITZERLAND

ABSTRACT

DEBEVEC, T., V. PIALOUX, I. B. MEKJAVIC, O. EIKEN, P. MURY, and G. P. MILLET. Moderate Exercise Blunts Oxidative Stress Induced by Normobaric Hypoxic Confinement. *Med. Sci. Sports Exerc.*, Vol. 46, No. 1, pp. 33–41, 2014. **Purpose**: Both acute hypoxia and physical exercise are known to increase oxidative stress. This randomized prospective trial investigated whether the addition of moderate exercise can alter oxidative stress induced by continuous hypoxic exposure. **Methods**: Fourteen male participants were confined to 10-d continuous normobaric hypoxia (F_IO_2 = 0.139 ± 0.003, P_IO_2 = 88.2 ± 0.6 mm Hg, ~4000-m simulated altitude) either with (HCE, n = 8, two training sessions per day at 50% of hypoxic maximal aerobic power) or without exercise (HCS, n = 6). Plasma levels of oxidative stress markers (advanced oxidation protein products [AOPP], nitrotyrosine, and malondialdehyde), antioxidant markers (ferric-reducing antioxidant power, superoxide dismutase, glutathione peroxidase, and catalase), nitric oxide end-products, and erythropoietin were measured before the exposure (Pre), after the first 24 h of exposure (D1), after the exposure (Post) and after the 24-h reoxygenation (Post + 1). In addition, graded exercise test in hypoxia was performed before and after the protocol. **Results**: Maximal aerobic power increased after the protocol in HCE only (+6.8%, $P < 0.05$). Compared with baseline, AOPP was higher at Post + 1 (+28%, $P < 0.05$) and nitrotyrosine at Post (+81%, $P < 0.05$) in HCS only. Superoxide dismutase (+30%, $P < 0.05$) and catalase (+53%, $P < 0.05$) increased at Post in HCE only. Higher levels of ferric-reducing antioxidant power (+41%, $P < 0.05$) at Post and lower levels of AOPP (−47%, $P < 0.01$) at Post + 1 were measured in HCE versus HCS. Glutathione peroxidase (+31%, $P < 0.01$) increased in both groups at Post + 1. Similar erythropoietin kinetics was noted in both groups with an increase at D1 (+143%, $P < 0.01$), a return to baseline at Post, and a decrease at Post + 1 (−56%, $P < 0.05$). **Conclusions**: These data provide evidence that 2 h of moderate daily exercise training can attenuate the oxidative stress induced by continuous hypoxic exposure. **Key Words:** PROOXIDANT, ANTIOXIDANT, NITRATIVE STRESS, CHRONIC NORMOBARIC HYPOXIA, PHYSICAL EXERCISE

BASIC SCIENCES

Both short-term (21,31) and long-term (1,10,16,40) exposures to hypoxia can result in oxidative damage as a consequence of increased reactive oxygen species (ROS) generation and a reduction of antioxidant defenses. The underlying mechanisms of the ROS production induced by decreased oxygen availability include an increase in catecholamine production (23), a decrease in mitochondria redox potential (17), and an activation of xanthine oxidase pathway (45).

Acute physical exertion, performed under normoxic (15) and hypoxic (3,24,29) conditions, also augments ROS production and results in increased oxidative stress (32). Exercise drastically enhances oxygen delivery to the working muscles. This increase of O_2 flux can lead to a mitochondrial overproduction of the superoxide anion ($O_2^{\cdot-}$) and oxygen-derived intermediates (37) that promote oxidative damage to biological molecules (14). Contrary to acute exercise, regular exercise training was shown to upregulate the antioxidant system and can lead to reduced overall oxidative stress levels at rest and in responses to acute exercise (15,27,35).

To date, studies investigating the effects of combined hypoxia and exercise on oxidative stress and antioxidant status have been mainly performed using different intermittent altitude training models, such as live-high train-low (LH-TL) and live-low train-high (LL-TH) (28,30,31). However, these training models have resulted in different pro-oxidant/antioxidant balance responses, likely depending on the hypoxic dose and/or the intensity of the training. Indeed, it has been shown that when performing high intensity exercise training sessions, both LH-TL (30) (250 h of cumulative hypoxic exposure) and LL-TH (29) (12 training sessions in hypoxia) significantly impair antioxidant capacities. On the other hand,

Address for correspondence: Tadej Debevec, Ph.D., Department of Automation, Biocybernetics and Robotics, Jozef Stefan Institute, Jamova 39, 1000, Ljubljana, Slovenia; E-mail: tadej.debevec@ijs.si.
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LH-TL (28) (210 h of cumulative hypoxic exposure) using low-intensity endurance training did not affect antioxidant status. It was thus suggested that low-intensity normoxic endurance training performed during the LH-TL may act as a preconditioning stimuli by reducing hypoxia-induced oxidative stress via enhanced cellular ROS defense (28). Such preconditioning effect might also occur during continuous normobaric hypoxic exposure, if moderate exercise training was performed in hypoxia (3,14). However, the influence of regular exercise training on chronic hypoxia-induced ROS overproduction and antioxidant defenses reduction has not yet been investigated.

Accordingly, the aim of the present study was to investigate the changes in oxidative stress markers during and after 10 d of continuous normobaric hypoxic exposure with or without daily moderate exercise training. We hypothesized that (i) 240 h confinement to a 4000-m simulated altitude is a sufficient hypoxic dose to increase oxidative stress and (ii) the addition of moderate aerobic exercise training could attenuate the oxidative stress induced by continuous hypoxic exposure, via upregulation of antioxidant systems.

METHODS

Participants. Healthy active male participants were initially invited to participate in the study through local advertisements. Exclusion criteria included smoking, cardiorespiratory diseases, and hematological and kidney disorders. To prevent preacclimatization bias, participants who were exposed to altitudes above 2000 m during the month before the onset of the study were also excluded. After initial screening, the selected participants were randomly and equally assigned to either the hypoxic exercise confinement (HCE group, $n = 8$) or hypoxic sedentary confinement (HCS group, $n = 8$). All participants were recreationally active (at least one to two aerobic exercise sessions per week) sea-level dwellers and gave a written informed consent before the start of the study. During the protocol, two participants from the HCS group were withdrawn from the study due to health issues (one due to acute appendicitis and the other due to general adaptation problems). Accordingly, eight participants in the HCE group ($n = 8$, age = 25.8 ± 2.4 yr, stature = 182.7 ± 5.9 cm, body mass = 76.6 ± 6.3 kg, body mass index = 22.9 ± 1.2 $kg \cdot m^{-2}$, body fat [BF] = 22.8% ± 6.2%, maximal oxygen consumption [$\dot{V}O_{2max}$] = 42.6 ± 6.1 $mL \cdot kg^{-1} \cdot min^{-1}$, maximal aerobic power during the cycle ergometer test [PPO] = 300.6 ± 38.1 W; mean ± SD) and six participants in the HCS group ($n = 6$; age = 24.8 ± 3.1 yr, stature = 177.7 ± 3.5 cm, body mass = 70.4 ± 10.0 kg, body mass index = 22.3 ± 2.5 $kg \cdot m^{-2}$, BF = 21.9% ± 4.7%, $\dot{V}O_{2max}$ = 42.2 ± 5.0 $mL \cdot kg^{-1} \cdot min^{-1}$, PPO = 281.3 ± 47.1 W) finished the whole protocol, and only their data were included in the final analysis.

Study design. This study was performed in a randomized fashion, using one independent factor (HCE group [hypoxic exposure + exercise training] vs HCS group [hypoxic exposure only]) and four repeated measures (four blood sampling periods [Pre: before the protocol; D1: after the first 24 h in hypoxia; Post: after the 10-d hypoxic confinement; Post + 1: 24 h after reexposure to normoxia]). The outline of the study design is presented in Figure 1. Both experimental procedures and the confinement took place in a normobaric hypoxic facility (Olympic Sports Center Planica, Slovenia; situated 940 m above sea level). The participants were accommodated in the hypoxic facility for at least 2 d before the start of the protocol for familiarization to the experimental environment and baseline testing. All experimental procedures were performed according to the Declaration of Helsinki. The National Ethics Committee at the Ministry of Health of the Republic of Slovenia approved the study.

Hypoxic confinement. During the 10-d experimental period, the participants were confined 24 $h \cdot d^{-1}$ to one floor of the Olympic Sport Centre. During this time, they were assigned to double bedrooms and were allowed to move freely in the hallway connecting the rooms (110 m^2). In addition, they shared a common living room and dining room in the hypoxic facility. The environmental conditions on the whole floor remained stable throughout the study (ambient temperature = 23.1°C ± 1.0°C, relative humidity = 56% ± 8%, and ambient pressure = 682 ± 4 mm Hg). For each participant, the hypoxic exposure commenced at 9:00 a.m. on day 1 and was terminated at 9:00 a.m. on day 11 (Post). The cumulative exposure of each individual was exactly 240 h (see Fig. 1). The normobaric hypoxic condition simulated an altitude of 4000 m. This was achieved by reducing the fraction of inspired O_2 (F_IO_2) in the ambient to 0.139 ± 0.003, resulting in a partial pressure of inspired O_2 (P_IO_2) of 88.2 ± 0.6 mm Hg. The reduction in F_IO_2 on the whole floor (10 rooms, hallway, living room, and dining area) was achieved

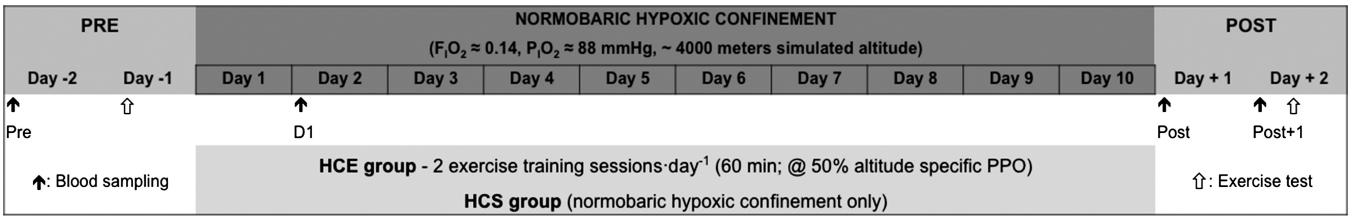

FIGURE 1—Study outline (F_IO_2, Fraction of inspired oxygen; P_IO_2, Partial pressure of inspired oxygen; PPO, maximal aerobic power during the cycle ergometer test).

and maintained by a vacuum pressure swing adsorption system (b-Cat, Tiel, the Netherlands) that generated and delivered oxygen-depleted air to the designated area. The F_IO_2 was monitored and adjusted, if necessary, throughout the experiment using designated O_2 sensors in each room, connected to the vacuum pressure swing adsorption control system. Before the analysis of the room air samples, which was repeated every 15 min throughout the 10-d confinement, the O_2 and the CO_2 analyzers were automatically calibrated using precision calibration gases (Messer, Ljubljana, Slovenia). For safety reasons, all participants wore a portable personal ambient O_2 analyzer (Rae PGM-1100; Rae Systems Inc., San Jose, CA) with an audible alarm throughout the experimental period. The same dietary menu, designed according to the recommendations for low nitrate/nitrite diet by Wang et al. (43), was applied to all participants throughout the whole protocol, including testing periods with the caloric ratio (%) of 60:25:15 for the percentage of carbohydrate, fat, and protein, respectively. Individual targeted caloric intake values were calculated by multiplying participants' resting energy expenditure with an activity factor of 1.4 for the HCS and 1.8 for the HCE group to account for the different activity levels (20). The participants were allowed to drink water and unsweetened tea *ad libitum*; however, they were encouraged to drink at least 2 L of fluid per day.

Exercise training. During the 10-d hypoxic confinement period, the HCE group participants performed 2 h of moderate-intensity exercise training daily (2 × 60 min) on a mechanically braked cycle ergometer. The first daily training sessions were performed in the morning (between 10:00 a.m. and 12:00 a.m.) and the second in the afternoon (between 3:00 p.m. and 5:00 p.m.). All training sessions were performed in a designated training laboratory under the same environmental conditions as the confinement area (F_IO_2 = 0.140 ± 0.004, P_IO_2 = 88.8 ± 0.6 mm Hg). During the training sessions, participants' HR and capillary oxyhemoglobin saturation (SpO_2) were constantly monitored using the iBody Application (iBody; Wahoo Fitness, Atlanta, GA) on an iPad (Apple, Cupertino, CA) and finger oximetry device (3100 WristOx; Nonin Medicals, Plymouth, MN), respectively. The training intensity was individually adjusted. The HR target corresponded to the HR measured at 50% of the altitude-specific maximal aerobic power (PPO) achieved during a graded exercise test to exhaustion conducted under hypoxic conditions during the baseline (Pre) testing period. The HR was constantly monitored and maintained within the individuals' targeted level (±4 beats per minute) by adjustment of the workload. The HCS group participants were not allowed to perform any type of dynamic or static exercise during the confinement period.

Experimental procedures. Stature, body mass, and BF of the participants were determined using a combined scale and stadiometer (Seca 703; Seca, Hamburg, Germany) and dual-energy x-ray absorptiometry scanning (Discovery W-QDR series; Hologic, Bedford, MA). The quantification of hypoxia effects during the confinement period on HR and SpO_2 was performed each morning in the supine position, before the participants getting out of bed, for at least 10 min using a finger oximetry device (3100 WristOx; Nonin Medicals). In addition, the self-assessment part of the Lake Louise mountain sickness questionnaire was completed daily by all participants to obtain the Lake Louise Score (LLS; 0–15) as an index of acute mountain sickness (AMS) occurrence and severity. AMS was defined as LLS ≥ 3 with concomitant headache.

Exercise testing. To assess baseline aerobic performance, participants performed an incremental test to exhaustion on an electrically braked cycle ergometer (Ergo Bike Premium; Daum Electronics, Fürth, Germany) before the start of the protocol. The protocol comprised a 2-min resting period, followed by a 2-min warm-up at 60 W. Thereafter, the workload was increased by 25 $W \cdot min^{-1}$ until task failure. The criteria for the attainment of $\dot{V}O_{2max}$ included a plateau in O_2 consumption, respiratory exchange ratio > 1.1, and inability to maintain the pedaling cadence higher than 60 rpm. $\dot{V}O_{2max}$ was expressed as the average of $\dot{V}O_2$ values recorded during the last 60 s of the test. A calibrated metabolic cart (Quark CPET; Cosmed, Rome, Italy) was used for breath-by-breath gas exchange measurement during the test. PPO was calculated as follows: $PPO = P_{compl} + (t(60 \times 25)^{-1})$, where P_{compl} corresponds to the last completed workload and t corresponds to the number of seconds during the final uncompleted workload.

Before and after the confinement, the participants also performed an incremental cycle ergometer test (Ergo Bike Premium; Daum Electronics) to exhaustion in normobaric hypoxia. The testing protocol comprised a 2-min resting period in normoxia, a 5-min resting period in hypoxia, followed by a 2-min warm-up at 60 W. Thereafter, the workload was increased 25 $W \cdot min^{-1}$ until task failure. During the test, participants inspired a humidified hypoxic gas mixture (F_IO_2 = 0.14) from a 200-L Douglas bag using a low resistance two-way valve (2700 NRBV; Hans Rudolph Inc., Shawnee, KS) and an oronasal mask (7920 AL; Hans Rudolph Inc.). PPO was calculated according to the formula described earlier. Continuous measurements of SpO_2 (BCI 3301; Nellcor, Boulder, CO) and HR (iBody; Wahoo Fitness) were performed during the tests. Participants also reported their ratings of perceived exertion on a modified Borg scale (0–10), separately for leg (RPE_{leg}) and dyspnea (RPE_{dys}) sensations.

Blood sampling and biochemical analyses. Venous blood samples (8 mL) were obtained before (Pre), after the first 24 h of hypoxic exposure (D1), immediately before return to normoxia after 240 h of hypoxic exposure (Post), and after the first 24 h back in normoxia (Post + 1), as shown in Figure 1. All blood samples were taken from an antecubital vein in the morning, before the participants stood up from the nocturnal supine position. A 2-mL blood sample was immediately transferred and analyzed in a nearby medical laboratory for hemoglobin concentration (Hb) and hematocrit (Hct) using an automated laser-based hematology analyzer (ADVIA 2120; Siemens, München, Germany). The remaining venous blood was centrifuged (10 min at 3500 rpm, 4°C), stored in

five 400-μL aliquots, and frozen at −80°C for subsequent analysis performed in a blinded manner, less than 6 months after the experimental protocol.

Erythropoietin (EPO) concentration was determined in plasma using sandwich enzyme-linked immunoassay (Human Erythropoietin Quantikine IVD ELISA; R&D Systems, Minneapolis, MN). The quantification of the optical density was performed on a microplate reader AD 340C (Beckman Coulter, Brea, CA) set at 450 nm and corrected at 600 nm. All samples were assayed in duplicate. The intra-assay coefficient of variation of the analysis was 5.0%.

Concentration of plasma advanced oxidation protein products (AOPP), nitrotyrosine as end product of protein nitration, malondialdehyde (MDA), ferric-reducing antioxidant power (FRAP), nitrite and nitrate (NOx) as marker of nitric oxide (NO) metabolism, and the activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase were measured as previously described (11). The intra-assay coefficient of variation was 5.4% for AOPP, 6.8% for nitrotyrosine, 2.2% for MDA, 2.9% for FRAP, 5.6% for SOD, 4.6% for GPX, 3.1% for catalase, and 5.4% for NOx.

Statistical analysis. Data are reported as mean ± SD, unless indicated otherwise. Differences between baseline group characteristics were analyzed using an independent-samples Student's *t*-test. The oxidative stress and antioxidant markers, hematological characteristics, exercise test outcomes, daily SpO_2, HR, and LLS scores were analyzed using a one-way repeated-measures unbalanced ANOVA. When ANOVA analysis revealed significant *F*-ratio for the main effect, a Tukey HSD *post hoc* test was used to define the specific differences. *Z* Fischer test correlations were used to analyze the relationship between different continuous variables. A *P* value of 0.05 was considered statistically significant. All statistical analyses were performed using Statistica 5.0 (StatSoft, Tulsa, OK). For the parameters with statistical differences, the observed statistical analysis power revealed by the repeated-measure ANOVA with an alpha set at 0.05 was 0.97 for LLS (time effect), 0.96 for HR (time–group effect), 0.85 for SpO_2 (time–group effect), 1.00 for EPO (time effect), 0.98 for Hb (time effect), 0.99 for Hct (time effect), 0.85 for AOPP (time–group effect), 0.68 for nitrotyrosine (time–group effect), 0.73 for FRAP (time–group effect), 0.85 for SOD (time–group effect), 0.87 for GPX (time effect), and 0.71 for catalase (time–group effect).

RESULTS

Hypoxic exposure and training effort. There were no significant differences between groups in physical characteristics, age, $\dot{V}O_{2peak}$, and PPO before the protocol. All participants tolerated the hypoxic confinement without significant adverse effects, except for one participant with acute appendicitis and one with general adaptation problems whose data were excluded from the analysis. As noted in Figure 2, a decrease in resting HR was significant in the HCE group

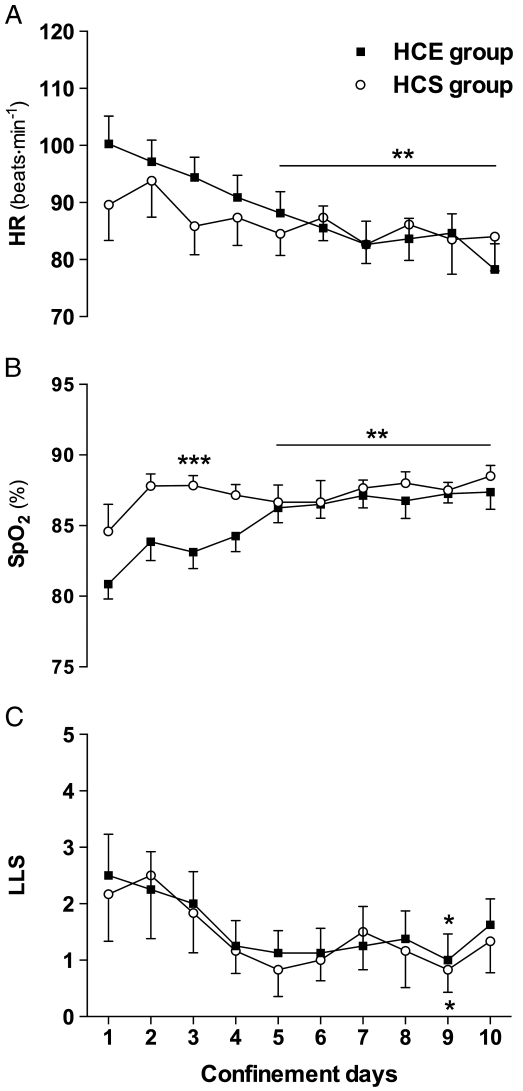

FIGURE 2—Resting heart rate (HR; A), capillary oxyhemoglobin saturation (SpO_2; B), and Lake Louise Score (LLS; C) (mean ± SEM) during the 10-d normobaric hypoxic confinement in both hypoxic exercise (HCE) and hypoxic sedentary (HCS) group. **$P < 0.01$ and *$P < 0.05$ significant difference compared with day 1; ***$P < 0.01$ significant difference between groups.

only ($P < 0.01$) from day 1 (HCE: 100 ± 12 beats per minute; HCS: 89 ± 14 per minute) to day 10 (HCE: 80 ± 12 per minute; HCS: 84 ± 14 beats per minute). The morning SpO_2 (Fig. 2) values were significantly lower in the HCE group compared with the HCS on day 3 of the confinement (HCE: 82.9% ± 3.3%; HCS: 87.7% ± 1.7%; $P < 0.01$). Overall there was a significant increase in the SpO_2 values in HCE group only from day 1 (80.9% ± 2.8%) to day 10 (87.3% ± 3.4%, $P < 0.01$). The LLS scores were comparable between groups and were significantly reduced in both groups on day 9 of the confinement compared with day 1 (Fig. 2). The average (range) LLS values throughout the confinement were 1.5 (0–6) and 1.4 (0–7) for the HCE and HCS group, respectively. The AMS was noted in four participants in the HCE and three participants in the HCS group at D1.

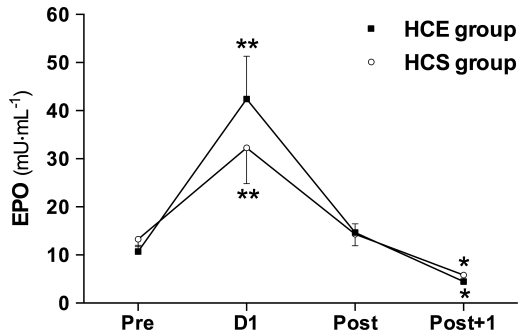

FIGURE 3—Plasma erythropoietin (EPO) concentration (mean ± SEM) before (Pre), after the first 24 h of hypoxic confinement (D1), after 10 d of continuous hypoxic confinement (Post), and after the first 24 h of reoxygenation (D + 1) in both hypoxic exercise (HCE) and hypoxic sedentary (HCS) group. **$P < 0.01$, *$P < 0.05$ significant difference compared with Pre.

The participants in the HCE group performed all 20 training sessions and reported no adverse effects. No changes were noted in HR and SpO_2 between different training sessions with average values of 144 ± 11 beats per minute and 80.2% ± 2.7% on day 1 and 141 ± 10 beats per minute and 83.4% ± 3.4% on day 10, respectively.

Hypoxic incremental cycle test. Following the protocol, PPO was significantly higher in the HCE group (259 ± 34 vs 276 ± 34 W, $P < 0.05$) and did not change in the HCS group (244 ± 37 vs 247 ± 41 W). Also, no changes were noted in the peak values of SpO_2, HR, RPE_{leg}, and RPE_{dys} after hypoxic exposure only (HCS group). A significant decrease in peak HR (184 ± 4 vs 177 ± 6 beats per minute, $P < 0.05$) and an increase in RPE_{dys} (7.9 [6–9] vs 8.6 [7–10], $P < 0.05$) were noted in the HCE group with no changes in SpO_2 and RPE_{leg}.

Hematological markers. Compared with baseline, plasma EPO concentration was significantly increased at D1 (HCE: +296%; HCS: +143%; $P < 0.01$) in both groups and decreased 24 h after reoxygenation at Post + 1 (HCE: −58%; HCS: −56%; $P < 0.05$) (Fig. 3). A significant increase in Hb (+7%, $P < 0.05$) and Hct (+7%, $P < 0.05$) was noted in both groups at Post only (Table 1).

Oxidative stress markers. AOPP (+28%, $P < 0.05$) and nitrotyrosine (+81%, $P < 0.05$) were increased only in the HCS group at Post + 1 and Post, respectively (Fig. 4) whereas no changes in MDA plasma concentration were noted in either group (Table 1). In addition, HCE group expressed lower AOPP concentration than HCS at Post + 1 (−47%, $P < 0.01$).

The HCE group significantly increased plasma SOD (+30%, $P < 0.05$) and catalase (+53%, $P < 0.05$) activities at Post (Fig. 4, Table 1), although these markers were not significantly affected at any time in HCS. Moreover, FRAP were significantly higher in the HCE compared with HCS group at Post (+41%, $P < 0.05$ for FRAP). Significant increases in GPX (+31%, $P < 0.01$) at Post + 1 were observed independently of the group (Table 1). No significant changes were noted in NOx.

Individual changes in nitrotyrosine were negatively correlated ($r = 0.49$, $P < 0.05$) to changes in LLS in the HCS group only. No significant correlation was noted between changes in other oxidative stress/antioxidant markers and changes in HR, SpO_2, LLS, EPO, Hb, and Hct.

DISCUSSION

The aim of the present study was to determine whether the addition of moderate exercise training to prolonged continuous normobaric hypoxic exposure alters oxidative stress responses compared with hypoxic exposure only. First, our data show that continuous 10-d hypoxic exposure to simulated altitude of 4000 m significantly increases oxidative stress as reflected in higher AOPP (+28%) and nitrotyrosine (+81%) following the protocol in the HCS group only. Second, our results demonstrate that the addition of daily moderate exercise training attenuates oxidative stress because HCE did not increase either AOPP or nitrotyrosine at the end of the hypoxic confinement. The increased SOD (+30%) and catalase (+53%) activities only in the HCE after the protocol suggest that moderate exercise training may

TABLE 1. Selected oxidative stress and hematological markers assessed before, during, and after the experimental protocol in both groups.

	Pre	D1	Post	Post + 1
HCE group				
MDA ($\mu mol \cdot L^{-1}$)	29.1 ± 23.4	34.9 ± 34.8	28.6 ± 20.9	30.9 ± 26.0
GPX ($\mu mol \cdot L^{-1} \cdot min^{-1}$)	11.29 ± 2.95	11.07 ± 2.05	11.21 ± 2.23	13.66 ± 1.38*
Catalase ($\mu mol \cdot L^{-1}$)	18.47 ± 11.75	16.34 ± 7.65	28.26 ± 14.23**	21.34 ± 9.77
NOx ($\mu mol \cdot L^{-1}$)	29.11 ± 4.17	27.66 ± 5.58	27.93 ± 8.88	32.69 ± 5.51
Hb ($g \cdot L^{-1}$)	151.6 ± 8.3	152.3 ± 10.8	162.0 ± 15.2**	150.3 ± 11.9
Hct (%)	0.44 ± 0.03	0.44 ± 0.03	0.47 ± 0.04**	0.44 ± 0.03
HCS group				
MDA ($\mu mol \cdot L^{-1}$)	32.8 ± 40.7	34.7 ± 40.3	22.1 ± 26.4	21.8 ± 25.2
GPX ($\mu mol \cdot L^{-1} \cdot min^{-1}$)	10.90 ± 0.99	12.03 ± 2.45	12.36 ± 2.37	15.99 ± 7.45*
Catalase ($\mu mol \cdot L^{-1}$)	23.55 ± 17.83	18.90 ± 11.63	25.15 ± 25.10	20.34 ± 20.25
NOx ($\mu mol \cdot L^{-1}$)	28.95 ± 8.21	26.46 ± 2.96	26.48 ± 4.00	32.59 ± 13.11
Hb ($g \cdot L^{-1}$)	155.5 ± 7.5	162.3 ± 6.7	166.0 ± 8.3**	157.2 ± 7.5
Hct (%)	0.45 ± 0.02	0.47 ± 0.03	0.48 ± 0.03**	0.45 ± 0.02

Values are presented as means ± SD. HCE, hypoxic exercise group ($n = 8$); HCS, hypoxic sedentary group ($n = 6$); MDA, malondialdehydes; GPx, glutathione peroxidase; NOx, blood nitric oxide metabolites; Hb, hemoglobin; Hct, hematocrit; Pre, before the protocol; D1, after the first 24 h in hypoxia; Post, after 10 d in hypoxia; Post + 1, 24 h after reexposure to normoxia.
*$P < 0.05$ significant change from Pre.
**$P < 0.01$.

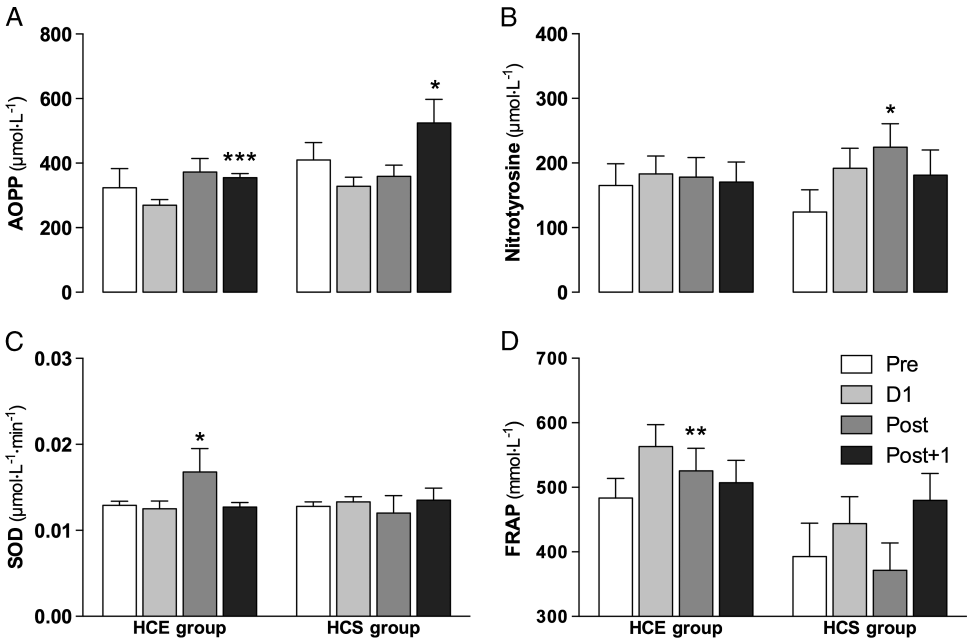

FIGURE 4—Plasma advanced oxidation protein products (AOPP; A), nitrotyrosine (B), superoxide dismutase (SOD; C), and plasma ferric-reducing antioxidant power (FRAP; D) values (mean ± SEM) before (Pre), after the first 24 h of hypoxic confinement (D1), after 10 d of continuous hypoxic confinement (Post), and after the first 24 h of reoxygenation (D + 1) in both hypoxic exercise (HCE) and hypoxic sedentary (HCS) group. *$P < 0.05$ significant difference compared with Pre; **$P < 0.05$ significant difference between groups; and ***$P < 0.01$.

lead to blunted oxidative stress through antioxidant defense improvement. This notion is further supported by the findings of significantly higher FRAP at Post, and lower AOPP at Post + 1 in the HCE than the HCS group, suggesting that the nonenzymatic antioxidant pool was preserved during chronic hypoxic exposure with addition of moderate-intensity exercise.

Increased oxidative stress in response to short-term (21,31) and long-term (1,10,16,40) hypoxic exposures at rest of different intensities is well established. Increased oxidative stress markers AOPP and nitrotyrosine after the 10-d confinement in the HCS group only confirm these observations. The findings of this study also confirm the reported reductions in antioxidant status after chronic hypoxic exposure (29,30) because FRAP was significantly lower in the HCS compared with the HCE group. Accordingly, the hypoxic dose used in this study (240 h at 4000-m simulated altitude) was of sufficient magnitude to increases oxidative stress and, at least transiently, impair the antioxidant reserves. Although the simulated altitude used in this study was relatively high, the exposure time was in line with the recommended minimal cumulative hypoxic exposure duration for erythropoiesis and ventilatory adaptation stimulation (220–250 h) during normobaric LH-TL protocols (34). The efficiency of the used hypoxic dose is reflected in increased hematological markers (Table 1, Fig. 3) and augmented SpO_2 observed during and after the confinement (Fig. 2).

Although the impairment of the antioxidant status is well documented after both acute hypoxia (1,21,24,29) and exercise (3,15,29,42), the possible interactions and combined effects on oxidative balance have, to date, received little attention. This is especially true for protocols using prolonged continuous hypoxic exposure combined with exercise. Because chronic exercise training under normoxia can be beneficial for cellular antioxidant system (9,15,25,35), these benefits might match the negative effects of intermittent hypoxia *per se*. This hypothesis has been recently suggested during 13 d of LH-TL in swimmers (28) and was also noted in a rodent model (13).

Indeed, our present findings indicate that regular moderate exercise, although performed under hypoxic condition, can attenuate continuous hypoxia-induced oxidative stress. This is especially interesting because it is well established that adding hypoxia to exercise significantly augments oxidative damage in certain circumstances (3,16,24,29,39). However, it should be noted that the studies reporting increased oxidative stress after hypoxic exercise were mainly based on high intensity exercises. The findings of the present study thus suggest that the intensity of the exercise plays a key role in the oxidative stress outcome, as previously suggested both for normoxia (26) and hypoxia (24,29). This is also in line with the findings of Marzatico et al. (22), reporting greater improvement of antioxidant enzymatic system after endurance training as compared with high-intensity training.

The potential oxidative benefits of moderate-intensity hypoxic exercise are supported by the augmentation of the antioxidant defense illustrated by increased SOD and catalase but also by the preservation of FRAP following the protocol in the HCE group (Fig. 4). The stimulation of SOD and catalase activities resulting from exercise training has likely buffered the ROS overproduction induced by hypoxia and thus limited the oxidative stress. Collectively, the

findings from the HCE group support the hypothesis that regular moderate exercise might counteract the hypoxia-induced oxidative stress (28). Moreover, our hypothesis is also supported by the negative correlation between changes of nitrotyrosine and LLS noted in the HCS group only. This observation also lends support to the hypothesis of Bailey et al. (4), suggesting that oxidative stress is likely involved in AMS.

Although Subudhi et al. (40) reported that prolonged submaximal exercise (55% hypoxic $\dot{V}O_{2max}$) in hypobaric hypoxia (4300 m) did not acutely increase the oxidative stress, our study revealed that exercise training attenuated oxidative stress induced by hypoxic exposure. The present study and that of Subudhi et al. were similar both as regard exercise intensity (50% vs 55%) and the level of hypoxia (4000 vs 4300 m). However, the difference in the magnitude of the exercise effect on oxidative stress can most probably be explained by the differences in the used exercise modalities (10 d of exercise training vs one acute exercise bout). This hypothesis is supported by the lack of oxidative stress change at D1 vs baseline in our study. The principal outcome of our study is that repeated endurance training under normobaric hypoxic conditions can decrease oxidative stress via antioxidant adaptation as previously shown for endurance training in normoxia (37,42). Interestingly, Subudhi et al. (40) did not observe any beneficial effect of 12 d of antioxidant supplementation on the oxidative stress induced by 2 wk of hypoxia exposure. Contrary to Subudhi et al. who supplemented their participants with a nonenzymatic antioxidants cocktail, our antioxidant stimulus was the result of physiological enzymatic adaptations (i.e., increase in SOD and catalase activities; Fig. 4 and Table 1) to exercise training. Taken together, these data suggest that moderate-intensity exercise training is more efficient in reducing oxidative stress than antioxidant supplementation, at least during continuous hypoxic exposure. Finally, it should to be noted that the study by Subudhi et al. was performed in hypobaric hypoxia, whereas normobaric hypoxia was used in our study. Despite our recent report (11) that hypobaric hypoxia induces higher ROS overgeneration than normobaric hypoxia, we currently do not know if the exercise-induced improvement in antioxidant defense would be different under hypobaric than under normobaric hypoxic condition.

Interestingly, nitrotyrosine was significantly increased in the HCS group at Post, immediately after reexposure to normoxia, whereas AOPP only increased 24 h after reoxygenation (Post + 1; Fig. 4). These data indicate that AOPP may be more sensitive to the reoxygenation phenomenon than to hypoxia *per se* as already observed in rodent model (2). This also suggests that activation of the xanthine oxidase pathway after hypoxia reoxygenation is involved in AOPP production as previously demonstrated in COPD patients (8).

We did not find any changes in MDA although previous studies reported an increase in MDA concentration after intermittent hypoxic training (29,31). The lack of observed changes in our study might be explained by a moderate sensitivity of the MDA assay to polyunsaturated fatty acid peroxidation (19). Consequently, it can be hypothesized that 10 d of continuous hypoxia is likely a less potent stimulus than 18 d of LH-TL using high-intensity exercise with respect to oxidative stress, in particular lipid peroxidation, despite a similar total hypoxic exposure (240 vs 250 h). The observed increase GPX at Post + 1 (Table 1) likely reflects the stimulation of antioxidant GPX enzymatic activity by increased oxygen availability after reoxygenation.

The average of almost three- and fourfold increases in plasma EPO concentrations for the HCS and HCE groups, respectively, were noted after the first 24 h of exposure to normobaric hypoxia (Fig. 3). The EPO kinetics observed in the study are in line with previous reports of initial increase and subsequent decrease of EPO after prolonged hypoxic exposure (18). Also, similarly to previous studies (12), a significant interindividual variability in the EPO response was noted in both the HCE and HCS group (increase range 50%–800%). No differences between groups were observed in EPO concentration at different sampling periods, although the increase tended to be higher in the HCE than the HCS group at D1. The observed downregulation of EPO and return of Hb and Hct levels to baseline at Post + 1 could be explained by the neocytolysis phenomenon, known to downregulate red cell mass after a large acute reduction in hypoxic stimulus as in high-altitude dwellers with polycythemia descending to sea level (33).

The insufficient nonenzymatic antioxidant dietary intakes have also been suggested to play an important role in stress responses to hypoxia and exercise (38,44). However, the differences in oxidative stress levels observed between groups in this study cannot be due to the differences in dietary antioxidant intakes because all participants were subjected to the same, individually adjusted diet throughout the experimental protocol. Furthermore, to limit the effect of dietary nitrate/nitrite intakes, the menu was designed according to the recommendations on reducing nitrate/nitrite intake (43).

It is known that hypoxic exposure in athletes interacts with their autonomic and cardiovascular adaptations to training (7). One cannot rule out that the higher resting HR during the first days compared with the second part of the confinement in the HCE group (Fig. 2) might result from exercise-induced residual fatigue. However, changes in resting HR are not consistently found in athletes experiencing overreaching (41) and are therefore considered as poor predictor of fatigue. One also cannot rule out that the cumulative effect of exercise and hypoxia on desaturation in the HEC group might have persisted after the exercise sessions and may therefore at least partly explain the lower saturation observed during the first days of the confinement (Fig. 2). However, although the level of physical activity is positively correlated to AMS (5), its relationship with resting SpO_2 (i.e., the maintenance of a lower saturation postexercise in hypoxia) is less clear. Schommer et al. (36) did not find any differences in resting SpO_2 at 5, 8, and 18 h

after moderate-intensity exercise in normobaric hypoxia ($F_IO_2 = 0.12$, ~4500-m simulated altitude) when compared with a nonexercise condition. It thus appears that the acclimatization was slowed during the first 4 d in the HEC group, but we cannot discern if this was the result of a residual exercise-induced fatigue, the maintenance of the exercise-induced desaturation, or other factors such as differences in sleep quality between the two groups.

The findings of this study not only have important implications for planning and modifying altitude-training modalities aiming for performance enhancement or weight loss but could also be potentially valuable in other settings. In particular, moderate- or low-intensity exercise could be used to reduce the hypoxia-induced oxidative stress during altitude sojourns or in clinical settings with patients suffering chronic hypoxia because of their pathological condition (e.g., sickle cell anemia, obstructive pulmonary diseases, etc.) as recently reported by Chirico et al. (6) in sickle-cell anemia patients. Indeed, the decrease of oxidative stress by regular physical training was shown to improve cardio- and cerebrovascular outcomes in a vulnerable population. In this context, it was suggested that physical training may reduce pathological outcomes of sleep-disordered breathing inducing intermittent-hypoxia by reducing oxidative stress (13).

In summary, this study is the first to demonstrate that adding moderate exercise sessions to normobaric hypoxic confinement attenuates hypoxia-induced oxidative stress. Our findings additionally suggest that this blunted oxidative stress is likely induced by an antioxidant system upregulation. Accordingly, future studies are warranted to further explore the possible oxidative stress controlling strategies using different intensities/doses of both exercise and hypoxia. In addition, the efficacy of endurance exercise leading to a better control of oxidative stress should be tested in diseases related to chronic or intermittent hypoxia.

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