Ventilation, Oxidative Stress, and Nitric Oxide in Hypobaric versus Normobaric Hypoxia

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ABSTRACT

FAISS, R., V. PIALOUX, C. SARTORI, C. FAES, O. DÉRIAZ, and G. P. MILLET. Ventilation, Oxidative Stress, and Nitric Oxide in Hypobaric versus Normobaric Hypoxia. *Med. Sci. Sports Exerc.*, Vol. 45, No. 2, pp. 253–260, 2013. **Purpose**: Slight differences in physiological responses and nitric oxide (NO) have been reported at rest between hypobaric hypoxia (HH) and normobaric hypoxia (NH) during short exposure. Our study reports NO and oxidative stress at rest and physiological responses during moderate exercise in HH versus NH. **Methods**: Ten subjects were randomly exposed for 24 h to HH (3000 m; F_IO_2, 20.9%; BP, 530 ± 6 mm Hg) or to NH (F_IO_2, 14.7%; BP, 720 ± 1 mm Hg). Before and every 8 h during the hypoxic exposures, pulse oxygen saturation (SpO_2), HR, and gas exchanges were measured during a 6-min submaximal cycling exercise. At rest, the partial pressure of exhaled NO, blood nitrate and nitrite (NOx), plasma levels of oxidative stress, and pH levels were additionally measured. **Results**: During exercise, minute ventilation was lower in HH compared with NH (−13% after 8 h, $P < 0.05$). End-tidal CO_2 pressure was lower ($P < 0.01$) than PRE both in HH and NH but decreased less in HH than that in NH (−25% vs −37%, $P < 0.05$). At rest, exhaled NO and NOx decreased in HH (−46% and −36% after 24 h, respectively, $P < 0.05$) whereas stable in NH. By contrast, oxidative stress was higher in HH than that in NH after 24 h ($P < 0.05$). The plasma pH level was stable in HH but increased in NH ($P < 0.01$). When compared with prenormoxic values, SpO_2, HR, oxygen consumption, breathing frequency, and end-tidal O_2 pressure showed similar changes in HH and NH. **Conclusion**: Lower ventilatory responses to a similar hypoxic stimulus during rest and exercise in HH versus NH were sustained for 24 h and associated with lower plasma pH level, exaggerated oxidative stress, and impaired NO bioavailability. **Key Words:** HIGH ALTITUDE, EXERCISE PHYSIOLOGY, NITRATE, NITRITE

BASIC SCIENCES

Exposure to hypoxia triggers rapid and important adaptive physiological responses. Hypoxic conditions can be defined as a combination of barometric pressure (BP) and an inspired fraction of oxygen (F_IO_2) that results in an inspired pressure of oxygen (P_IO_2) lower than a normoxic value of 150 mm Hg (5). The subsequent diminished arterial oxygen pressure (PaO_2) induces an increased pulmonary ventilation ($\dot{V}_E$) to maintain O_2 delivery to the tissues (42). It has been suggested that exposure to HH or normobaric hypoxia (NH) inducing the same P_IO_2 may elicit different physiological responses (5,18,25,28,36).

Savourey et al. (36) reported greater breathing frequency (f), lower tidal volume (V_t), and $\dot{V}_E$ in HH compared with NH during a 40-min acute hypoxic exposure at rest. Similar differences in $\dot{V}_E$ and V_t between HH and NH have been shown during 2 h at 4750 m (40) or during 10 h at 4770 m (25). However, the differences in cardioventilatory responses to exercise between HH and NH during longer exposure (i.e., more than a few hours) are not known.

Nitric oxide (NO) plays a significant role in the physiological responses to hypoxia. The partial pressure of exhaled NO (exNO) was shown to decrease at high altitude (4,8), although the putative effect of this decrease on pulmonary vasoconstriction remains debated (8,9). Accordingly, recent work reported lower levels of NO in exhaled air when measured at high altitude (HH) compared with an equivalent simulated altitude (NH) (18). The mechanisms underlying the observed differences are not known. Oxidative stress is a potential candidate.

Hypoxic exposure increased oxidative stress (33). Oxidative stress is known (i) to decrease the NO bioavailability in the vasculature (39) and (ii) to increase the acute ventilatory response to hypoxia (31) after chronic hypoxic exposure. However, to date, differences in oxidative stress between HH and NH have never been studied.

The aim of the present study was therefore to extend previous work demonstrating different ventilatory and

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nitrosative responses during short duration exposure in HH versus NH by determining if putative changes in ventilation and NO metabolism for 24 h at 3000 m in HH versus NH could be associated with different oxidative stress and pH variations.

METHODS

Subjects. Ten healthy lowland well-trained men (35 ± 8 yr; 179 ± 7 cm; 74 ± 8 kg; $\dot{V}O_{2max}$, 60.9 ± 6.6 $mL \cdot kg^{-1} \cdot min^{-1}$; range, 48–70 $mL \cdot kg^{-1} \cdot min^{-1}$) active in recreational and endurance sports (2-5 training sessions per week) participated in the study.

Volunteers signed an informed written consent. Subjects were all nonsmokers and neither acclimatized nor recently exposed to altitude. The experiment was approved by the local Medical Ethics Committee (CVEM, agreement 051/09, Sion, Switzerland) and performed in accordance with the Declaration of Helsinki.

Study design. Experimental design consisted of two different 24-h periods of exposure to HH and NH, in a random order with two groups of three subjects and one group of four subjects. For each subject, experiments were separated by 23 d in average (range, 15–32), allowing sufficient elimination of any acclimatization effect.

Measurements at altitude were taken either at 3000 m in a quiet and warmed room in the mountains (HH: F_IO_2, 20.9 ± 0.04%; BP, 530 ± 6 mm Hg; P_IO_2, 102 ± 0.3 mm Hg; temperature [*T*], 25°C ± 2°C; Col des Gentianes, Nendaz, Switzerland) or in a hypoxic chamber (ATS Altitude, Sydney, Australia) built in our laboratory (NH: F_IO_2, 14.7 ± 0.13%; BP, 720 ± 1 mm Hg; P_IO_2, 99 ± 0.4 mm Hg; *T*, 27°C ± 2°C; Sion, Switzerland). Normoxic measurements were completed outside the hypoxic chamber at an altitude of 485 m (F_IO_2, 20.9%; BP, 720 ± 1 mm Hg; P_IO_2, 140 ± 1.2 mm Hg; *T*, 24°C ± 1°C; Sion, Switzerland). BP and F_IO_2 were controlled every hour using precise electronic oximeter (GOX 100; Greisinger, Regenstauf, Germany) and barometer (GPB 2300, Greisinger), respectively.

Before any hypoxic trial, participants performed an incremental test with 3-min exercise steps on a cycle ergometer (Ergoline 900; Sensormedics) to determine maximal aerobic power (P_{max}) and maximal oxygen consumption ($\dot{V}O_{2max}$) using a portable indirect calorimeter (Metamax 3B; Cortex, Leipzig, Germany) (41). Gas exchanges were measured breath by breath with a portable indirect calorimeter (Metamax 3B; Cortex) calibrated immediately before each test using certified commercial gas preparation and a 3-L syringe. HR was measured continuously using a telemetry monitor (Polar Electro, Oy, Finland).

Pulse oxygen saturation (SpO_2) was recorded continuously at 0.25 Hz at the finger (Wristox 3100™ with 8000SM-WO Sensor; Nonin, Plymouth, MN) and averaged for the last 3 min preceding each moderate exercise (in seated position, at rest) and the last 3 min of each 6-min cycling bout (at exercise).

Experimental procedure. Five testing sessions were performed in HH and NH: PRE—baseline testing in normobaric normoxic condition in the morning before exposure to altitude; H + 1, H + 8, H + 16, and H + 24—measurement after 1, 8, 16, and 24 h of exposure at altitude, respectively.

In HH condition, H + 1 measures were performed upon arrival after 1 h of travel by car and cable car between the laboratory (485 m) and the building at altitude (3000 m) approximately 2 h after PRE measurement, corresponding to an exposure of approximately 45 min at an altitude higher than 2000 m and 30 min at 3000 m. In NH condition, H + 1 measures were performed after 60 min in the hypoxic chamber where the corresponding altitude was 2000 m on entering and 3000 m (F_IO_2, 14.7%) after 15 min.

The same nitrate- and nitrite-free meals and drinks, standardized for nutritional and caloric content, were provided to the subjects and ingested after H + 1, 90 min before H + 8, and 180 min before H + 24. Twenty-four hours before each trial, subjects were asked to refrain from any physical training and caffeine intake and reported their food intake (being replicated before the second trial). They were instructed to follow a low nitrate/nitrite diet for 4 d, avoiding fruits, salads, and cured meats as recommended by Wang et al. (43). Subjects started each 24-h hypoxic exposure exactly at the same time at approximately 1:00 p.m. They were allowed to talk, read, watch television and shortly walk inside the room but did not have any other physical activity than during the tests. Sleep time was asked to be of 8 h, but only within the 10:00 p.m. to 9:00 a.m. timeframe.

On the basis of previous research (27), we estimated that the prevalence of acute mountain sickness (AMS) would be relatively low (<13%) at the altitude of the experiment (3000 m) with our subjects exposed effortless to each hypoxic condition and exercising moderately only during 4 × 6 min for 24 h. Consequently, we did not include an evaluation of AMS symptoms. However, few subjects (2 of 10) reported some symptoms of light discomfort (e.g., headache and dizziness) in HH whereas none in NH. Because this difference might be of clinical relevance, future research at this altitude or higher should definitely assess the AMS severity.

Measurements at rest and during a 6-min submaximal cycling exercise in HH or NH condition at PRE, H + 1, H + 8, H + 16, and H + 24 were as follows.

HR and gas exchanges. Subjects were equipped with an oronasal mask (Vmask™, 7500 series; Hans Rudolph Inc., Shawnee, KS; dead space, 41 mL) to measure gas exchange with the same analyzer than during preliminary testing. This device measures volume using a bidirectional digital turbine. Oxygen uptake and carbon dioxide production are then determined in inspired and expired air successively with an electrochemical cell and an infrared analyzer, respectively, from the air drawn through a Nafion® sampling tube attached to the turbine at the output of the mask. F_IO_2 and F_ICO_2 are continuously measured to account for

putative deviation in ambient conditions. Distorted O_2 and CO_2 measurements for two subjects were excluded because of the defective calibration of the gas sensor before measurement. HR was recorded (RS800; Polar Electro). After a 6-min seated measurement period in a quiet environment, subjects started pedaling for 6 min on the subject-adjusted bicycle ergometer at a workload equal to 50% of their previously determined P_{max}. Parameters at exercise were calculated as the average of the last 3 min of the 6-min cycling bout. At the fifth minute of exercise, the rate of perceived exertion was evaluated using a 6–20 Borg scale.

Blood pressure. Blood pressure was measured at rest seated using an automatic device (BP A100; Microlife AG, Wildnau, Switzerland). Systolic blood pressure and diastolic blood pressure were manually reported.

Biochemical Analyses

Exhaled NO. The fraction of NO in the exhaled air was measured in standing position with a handheld electrochemical analyzer (NIOX MINO®; Aerocrine, Solna, Sweden) (19) following standard quality criteria (1) and a previously published procedure (8). For comparison across conditions, the fraction of exNO displayed by the device (ppb) was expressed as the partial pressure of exNO (nm Hg) (8). According to recent recommendations for measurements at altitude (17, 20), data obtained in HH at 3000 m were additionally corrected by applying a correction factor to adjust for the mass flow deviation and the higher sensitivity of the sensor at high altitude (see Table 2 in Donnelly et al. (8) and Table 1 in Hemmingsson et al. [17]).

Plasma nitric oxide, pH level, oxidative stress, and enzymatic antioxidants. A 5-mL blood sample was taken at rest from the antecubital vein at PRE, H + 1, H + 8, H + 16, and H + 24. After centrifugation, 400-μL aliquots of plasma were immediately frozen and stored at −80°C until blinded analysis less than 6 months after the experiment in the same laboratory. This warrants the perfect reproducibility of the analytic methods. Plasma total NO end products (NOx) (nitrate + nitrite + nitrosothiols) were measured with a chemiluminescence NO analyzer (Sievers 280 NOA; General Electric, Boulder, CO) after reduction of NOx to NO using VCl_3 in hydrochloric acid at 90°C (3) as previously performed in our laboratory (6).

The plasma pH level was measured at 37°C using the laboratory pH meter inoLab 720 (WTW, Weilheim, Germany). The accuracy of this pH meter is ± 0.004 (according to the manufacturer).

Plasma advanced oxidation protein products (AOPPs) were measured according to the semiautomated methods developed by Witko-Sarsat et al. (44). The plasma concentrations were determined by spectrophotometry and were calibrated with a chloramine-T solution that absorbs at 340 nm in the presence of potassium iodide. The absorbance of the reaction was read at 340 nm. AOPP concentrations were expressed as micromole per liter of chloramine-T equivalents. The intra-assay coefficient of variation is 5.4%.

The quantitative determination of the superoxide dismutase (SOD) activity was performed using the method described by Oberley and Spitz (29). SOD activity was determined by the degree of inhibition of the reaction between superoxide radicals, produced by a hypoxanthine–xanthine oxydase system and nitroblue tetrazolium. The intra-assay coefficient of variation is 5.6%.

Glutathione peroxidase (GPX) activity was determined by the modified method of Paglia and Valentine (30) as the rate of oxidation of NADPH to $NADP^+$ after addition of glutathione reductase (GR) and reduced glutathione (GSH) and NADPH, using H_2O_2 as a substrate. The intra-assay coefficient of variation is 4.6%.

Concentrations of plasma malondialdehyde (MDA), as thiobarbituric reactive substances, were determined as previously described (34). The pink chromogen was extracted with *n*-butanol, and its absorbance was measured at 532 nm by spectrophotometry using 1,1,3,3-tetraethoxypropan as standard. The intra-assay coefficient of variation is 2.2%.

Concentrations of plasma nitrotyrosine, as end product of protein nitration by $ONOO^{\cdot -}$, were measured by ELISA as previously described (12). The intra-assay coefficient of variation is 6.8%

Our research team routinely performs oxidative stress and antioxidants measurements as previously published (32).

Data Analysis and Statistics

Data are reported as means and standard deviations. Data were tested for normality (Shapiro–Wilk test) and equality of variance (Fisher–Snedecor *F*-test). When both conditions were met, a one-way ANOVA for repeated measures was performed for each condition (HH and NH) for the time effect with all pairwise multiple comparison procedures (Holm–Sidak method). Differences to baseline between HH and NH at the same time were then compared using a paired *t*-test. When normality or equality of variance were not met, variables were analyzed in each condition using a Friedman test for repeated measures on ranks for the time effect with all pairwise multiple comparison procedures (Bonferroni test). In this case, differences to baseline between NH and HH at identical times were then compared using a Mann–Whitney rank sum test. Null hypothesis was rejected at $P < 0.05$. All analyses were made using Sigmaplot 11.0 software (Systat Software, San Jose, CA).

RESULTS

Maximal aerobic power (P_{max}) determined during preexperimental tests was P_{max} (354 ± 50 W), ranging from 280 to 420 W.

Ventilatory and cardiovascular parameters. At rest, minute ventilation ($\dot{V}_E$) increased significantly in HH only after 1 h (+24%, $P < 0.05$) and in NH after 1, 8, and

TABLE 1. Time course of measured parameters at rest for 24 h in HH and NH.

Variable	Condition	PRE	H + 1	H + 8	H + 16	H + 24
SpO_2 (%)	HH	98 ± 1	93 ± 1**	91 ± 3**	92 ± 2**	93 ± 2**
	NH	97 ± 1	90 ± 3**	91 ± 2**	91 ± 2**	92 ± 1**
SBP (mm Hg)	HH	126 ± 8	124 ± 9	124 ± 9	121 ± 9	131 ± 10
	NH	126 ± 10	129 ± 13	123 ± 7	118 ± 9	129 ± 9
DBP (mm Hg)	HH	75 ± 9	75 ± 5	78 ± 9	77 ± 7	76 ± 6
	NH	81 ± 8	74 ± 5	77 ± 6	77 ± 7	77 ± 7
HR (bpm)	HH	57 ± 7	62 ± 8	68 ± 13*	61 ± 10	65 ± 9
	NH	57 ± 9	63 ± 10	69 ± 13*	66 ± 7	71 ± 10*
$\dot{V}_E$ ($L \cdot min^{-1}$)	HH	11.0 ± 1.8	13.6 ± 1.8*	11.8 ± 1.9#	10.7 ± 1.8†	12.7 ± 2.3#
	NH	10.3 ± 1.6	13.3 ± 3.3*	14.9 ± 3.5*	12.2 ± 1.6*	14.2 ± 1.5*
V_t ($L \cdot min^{-1}$)	HH	0.75 ± 0.15	0.88 ± 0.21	0.75 ± 0.21†	0.75 ± 0.23#	0.86 ± 0.25†
	NH	0.76 ± 0.18	0.89 ± 0.26	0.94 ± 0.3*	0.84 ± 0.24	0.95 ± 0.23*
f ($L \cdot min^{-1}$)	HH	15.5 ± 3.1	16.8 ± 3.4	16.8 ± 2.7	16.1 ± 3	16.8 ± 3.8
	NH	14.3 ± 2.7	15.9 ± 4.2	17.1 ± 4.4	15.8 ± 3.7	16.2 ± 3.8
$P_{ET}O_2$ (mm Hg) (n = 8)	HH	97.6 ± 4.0	66.4 ± 4.1**	61.9 ± 6.0**	65.0 ± 5.4**	65.6 ± 5.5**
	NH	98.6 ± 2.8	62.3 ± 2.8**	61.6 ± 2.2**	62.7 ± 2.6**	65.6 ± 2.8**
$P_{ET}CO_2$ (mm Hg) (n = 8)	HH	38.5 ± 3.5	33.4 ± 2.5*,#	33.8 ± 2.1*,††	33.1 ± 1.3*,††	30.8 ± 1.4*,††
	NH	36.8 ± 1.4	29.4 ± 2.4*	27.5 ± 1.3*	27.9 ± 0.9*	26.5 ± 1.5*
$\dot{V}O_2$ (mL $O_2 \cdot min^{-1} \cdot kg^{-1}$) ($n$ = 8)	HH	4.6 ± 0.6	6.7 ± 1.2	7.0 ± 1.2*	5.9 ± 0.4*	5.9 ± 1.2
	NH	4.5 ± 0.6	5.4 ± 0.9	6.1 ± 1.2*	5.1 ± 0.4	5.4 ± 0.6
$\dot{V}CO_2$ (mL $O_2 \cdot min^{-1} \cdot kg^{-1}$) ($n$ = 8)	HH	3.5 ± 0.7	5.2 ± 0.9	4.9 ± 1.0	4.6 ± 0.4	4.4 ± 1.2
	NH	3.7 ± 0.7	4.1 ± 0.8	4.2 ± 0.8	3.9 ± 0.2	4.1 ± 0.6
RER (n = 8)	HH	0.76 ± 0.14	0.78 ± 0.06	0.71 ± 0.03	0.77 ± 0.02	0.74 ± 0.07
	NH	0.82 ± 0.05	0.76 ± 0.06	0.70 ± 0.05*	0.76 ± 0.06	0.76 ± 0.04*
Displayed exNO (ppb)	HH	25 ± 14	27 ± 14	25 ± 15	22 ± 13	25 ± 15
	NH	23 ± 13	22 ± 14	21 ± 11	22 ± 13	23 ± 9
Corrected exNO (nm Hg)	HH	16.9 ± 9.8	9.5 ± 5.0**,††	8.8 ± 5.3**,††	7.9 ± 4.5**,††	8.9 ± 5.4**,††
	NH	15.2 ± 8.5	14.9 ± 9.2	14.1 ± 7.4	14.7 ± 8.6	15.7 ± 8.7
NOx (μM)	HH	40.6 ± 25.1	31.6 ± 19.6††	28.1 ± 18.9††	24.2 ± 16.3*,††	22.85 ± 16.2*,††
	NH	29.6 ± 9.7	27.7 ± 7.3	32.7 ± 9.7	30.2 ± 7.1	28.9 ± 6.9
GPX (% baseline)	HH	100	114 ± 26	85 ± 27	105 ± 43	103 ± 41
	NH	100	111 ± 30	123 ± 23	107 ± 21	121 ± 33
MDA (% baseline)	HH	100	117 ± 40	103 ± 62	111 ± 56	108 ± 52
	NH	100	92 ± 36	111 ± 35	116 ± 55	97 ± 51
Nitrotyrosine (% baseline)	HH	100	86 ± 16	77 ± 35	91 ± 20	75 ± 40
	NH	100	105 ± 26	75 ± 37	98 ± 16	87 ± 25

Values are presented as mean ± SD. n = 10 unless otherwise stated.
SpO_2, pulse oxygen saturation; SBP, systolic blood pressure; DBP, diastolic blood pressure; $\dot{V}_E$, minute ventilation (BTPS); V_t, tidal volume; f, breathing frequency; $P_{ET}O_2$, end-tidal O_2 pressure; $P_{ET}CO_2$, end-tidal CO_2 pressure; $\dot{V}O_2$, relative oxygen uptake; $\dot{V}CO_2$, relative carbon dioxide produced; RER, respiratory exchange ratio; exNO, exhaled NO; NOx, blood NO metabolites; GPX, glutathione peroxidase; MDA, malondialdehyde.
* * $P < 0.01$, * $P < 0.05$ for difference with PRE.
†† $P < 0.01$, † $P < 0.05$, # $P < 0.1$ for difference with NH.

24 h (+29%, +45%, and +38%, respectively, $P < 0.05$). Tidal volume (V_t) increased significantly only in NH after 8 and 24 h (+24% and +25%, respectively, $P < 0.05$). Eupneic end-tidal CO_2 pressure ($P_{ET}CO_2$) decreased immediately after 1 h in HH and NH (−13% and −20%, $P < 0.05$, respectively) and remained lower than baseline until 24 h in both conditions. Parameters at the different times at rest are summarized in Table 1.

TABLE 2. Time course of measured parameters during moderate exercise in HH and NH.

Variable	Condition	PRE	H + 1	H + 8	H + 16	H + 24
HR (bpm)	HH	116 ± 6	127 ± 7**	134 ± 11**	129 ± 9**	133 ± 8**
	NH	117 ± 9	130 ± 6**	138 ± 9**	133 ± 7**	136 ± 6**
f ($L \cdot min^{-1}$)	HH	26.8 ± 7.9	31.0 ± 6.0	34.0 ± 6.9*	32.0 ± 7.7*	32.8 ± 8.4*
	NH	24.1 ± 2.9	31.2 ± 6.6*	33.4 ± 6.3*	31.6 ± 6.9*	34.2 ± 6.5*
$P_{ET}O_2$ (mm Hg) (n = 8)	HH	97.0 ± 3.4	64.9 ± 2.5**	66.1 ± 4.1**	66.2 ± 2.4**	66.1 ± 4.4**
	NH	95.1 ± 3.5	65.0 ± 1.5**	67.0 ± 2.9**	65.9 ± 3.4**	67.8 ± 3.4**
$\dot{V}O_2$ (mL $O_2 \cdot min^{-1} \cdot kg^{-1}$) ($n$ = 8)	HH	34.0 ± 7.7	37.0 ± 6.3	41.4 ± 5.0§	40.5 ± 5.2*	40.9 ± 5.3§
	NH	36.2 ± 5.7	37.4 ± 5.0	39.2 ± 5.6*	38.0 ± 4.4*	39.3 ± 4.3*
$\dot{V}CO_2$ (mL $CO_2 \cdot min^{-1} \cdot kg^{-1}$) ($n$ = 8)	HH	32.2 ± 7.0	31.1 ± 8.2	35.8 ± 6.2§	35.5 ± 8.0§	35.0 ± 4.9
	NH	33.2 ± 5.8	31.9 ± 5.3	32.3 ± 5.2	32.3 ± 4.2	32.5 ± 3.5
RER (n = 8)	HH	0.95 ± 0.03	0.83 ± 0.09*	0.86 ± 0.06#	0.87 ± 0.11	0.85 ± 0.03*
	NH	0.92 ± 0.04	0.82 ± 0.06*	0.82 ± 0.05*	0.85 ± 0.06*	0.83 ± 0.03*
SpO_2 (%)	HH	97.7 ± 0.7	82.6 ± 4.2**	78.6 ± 5.5**	82.1 ± 4.6**	82.4 ± 4.1**
	NH	97.8 ± 0.8	83.1 ± 3.4**	78.1 ± 3.3**	81.5 ± 2.0**	82.6 ± 3.2**
RPE	HH	11 ± 1	13 ± 1*	14 ± 1 *†	13 ± 1*	13 ± 1*
	NH	11 ± 1	13 ± 1*	15 ± 1*	13 ± 1*	13 ± 1*

Values are presented as mean ± SD. n = 10 unless otherwise stated.
f, breathing frequency; $P_{ET}O_2$, end-tidal O_2 pressure; $\dot{V}O_2$, relative oxygen uptake; $\dot{V}CO_2$, relative carbon dioxide produced; RER, respiratory exchange ratio; SpO_2, pulse oxygen saturation; RPE, rate of perceived exertion (6–20 Borg scale).
* * $P < 0.01$, * $P < 0.05$, § $P < 0.1$ for difference with PRE.
† $P < 0.05$, # $P < 0.1$ for difference with NH.

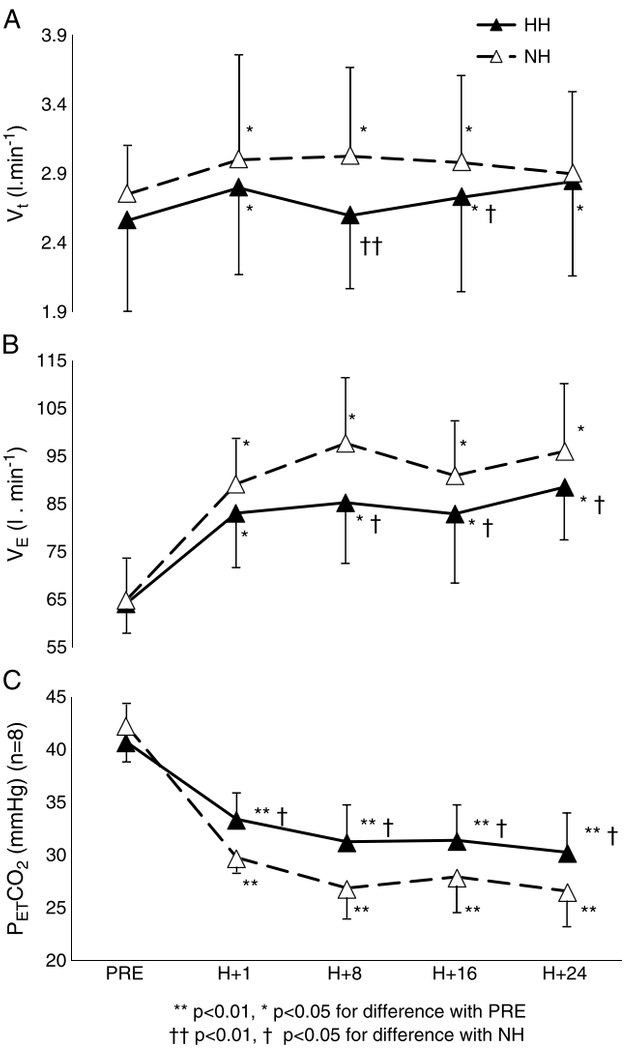

FIGURE 1—Tidal volume (V_t, L·min^{-1} [BTPS]) (A), minute ventilation ($\dot{V}_E$, lL·min^{-1} [BTPS]) (B), and end-tidal CO_2 pressure ($P_{ET}CO_2$, mm Hg) (C) during moderate exercise in HH and NH. n = 10 unless otherwise stated.

During moderate exercise, $\dot{V}_E$ and V_t were lower in HH compared with NH after 8 and 16 h ($P < 0.05$) (Figs. 1A and B). $P_{ET}CO_2$ was lower ($P < 0.01$) than PRE both in HH and NH after 1 h until 24 h (Fig. 1C). In addition, $P_{ET}CO_2$ decrease was lower in HH than that in NH (−25% vs −37%, $P < 0.05$).

HR during exercise was not significantly different between HH and NH and was higher than PRE from H + 1 to H + 24 ($P < 0.05$). Breathing frequency, end-tidal O_2 pressure, and SpO_2 were not significantly different in HH and NH, both at rest and during exercise. Parameters at the different times during moderate exercise are summarized in Table 2.

Nitric oxide. During the 24-h exposure, exNO decreased ($P < 0.01$) in HH (−43%, −49%, 53%, and −46% after 1, 8, 16, and 24 h, respectively) but remained stable in NH (Fig. 2A). Compared with NH, exNO was lower in HH after 1 h (−36%, $P < 0.01$), 8 h (−38%, $P < 0.01$), 16 h (−46%, $P < 0.01$), and 24 h (−43%, $P < 0.05$).

Similarly, NO_x decreased during the 24 h in HH (−37% at 24 h, $P < 0.01$) while stable in NH (+1%, NS) (Fig. 2B).

Oxidative stress, enzymatic antioxidants, and pH levels. During the 24-h exposure, AOPPs were higher ($P < 0.05$) in HH (+120% and +260% after 1 and 24 h, respectively) than those in NH (+13% and +88% after 1 and 24 h, respectively) (Fig. 3A). SOD was significantly higher in HH than that in NH after 24 h (37%, $P < 0.01$) (Fig. 3B).

The plasma pH level was stable in HH but increased in NH ($P < 0.01$) where it remained higher than HH ($P < 0.01$) until 24 h (Fig. 3C).

DISCUSSION

Here we show for the first time different responses to an equivalent hypoxic stimulus in HH versus NH sustained for 24 h and associated with impaired NO bioavailability and exaggerated oxidative stress.

First, the acute ventilatory response to hypoxia (AHVR), usually associated with rapid ascents to high altitude (21), was lower in HH compared with NH in our study, in agreement with previous observations done during shorter hypoxic exposures (36).

Second, lower amounts of NO in the exhaled air (exNO) have also been reported in HH compared with NH and ascribed to differences in barometric pressure (10,18,20). Accordingly, our data show significantly less exNO in HH compared with NH. By comparison with the results of Hemmingson et al. (18) who reported lower exNO in HH

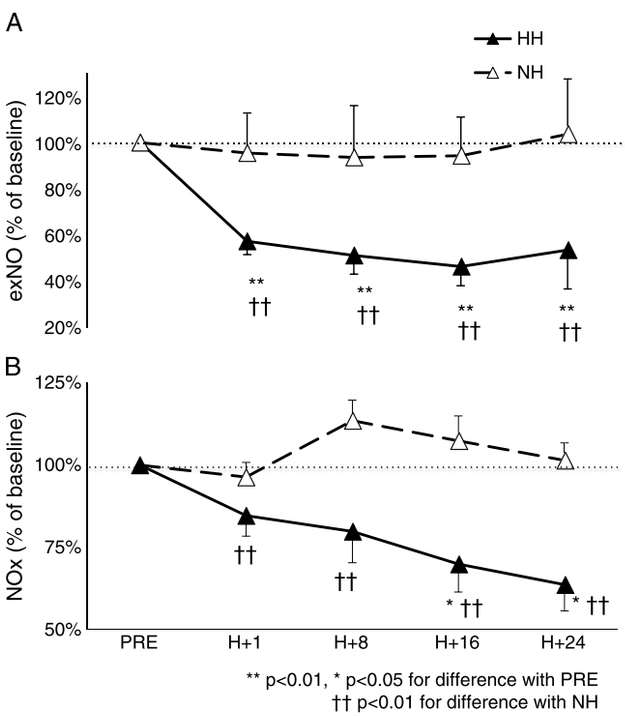

FIGURE 2—exNO (% baseline) (A) and blood NO metabolites (NOx, % baseline) (B) at rest during the 24 h in HH and NH.

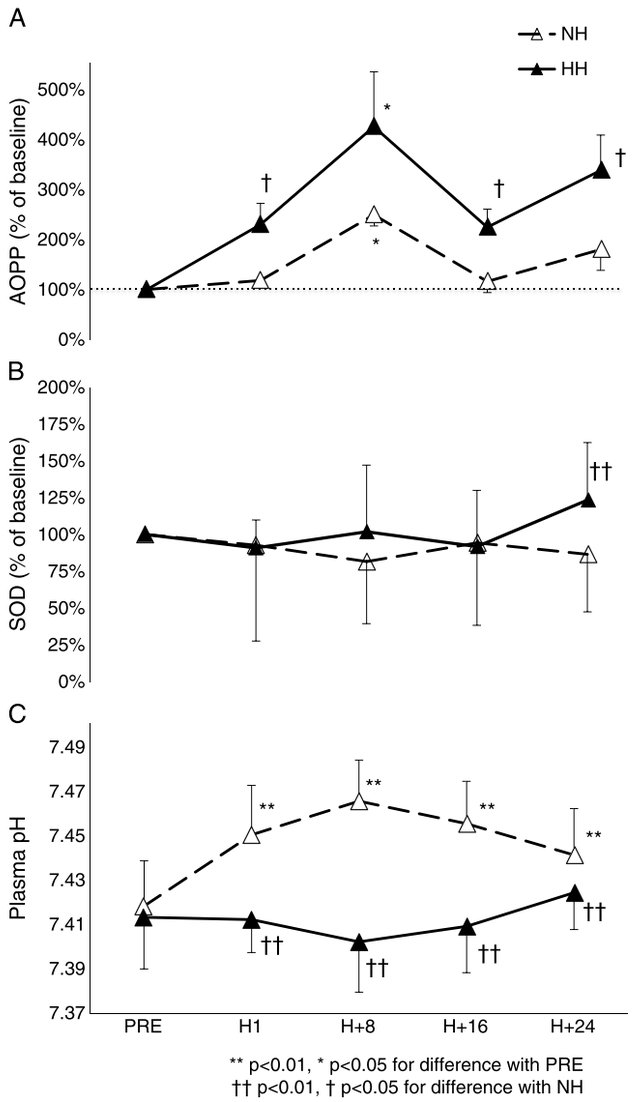

FIGURE 3—Blood AOPPs (% baseline) (A), SOD (% baseline) at rest during the 24 h in HH and NH (B), and plasma pH level at rest during the 24 h in HH and NH (C).

than that in NH during an acute exposure to very high altitude (10 min at 5000 m), the present data additionally show that the exNO can decrease in HH up to 24 h of exposure at an altitude of 3000 m. As proposed, the increased axial back diffusion of NO in the alveolar compartment when BP is decreased (37) coupled with the strong affinity of NO to hemoglobin (23) or pressure-induced suppression of the NO formation in the airways (20) may explain the lower exNO observed in HH. However, exNO should be interpreted with care because values were corrected for flow deviation and sensor sensitivity with correction factors of Hemmingsson et al. (17), which may vary due to different subject and/or sensor. Nevertheless, our results are in accordance with previously published results (18) but suggest other mechanisms for differences in ventilatory responses between HH and NH based on the interplay between exaggerated oxidative stress and impaired NO bioavailability in blood and tissue.

Indeed, the important new point is that plasma NO (i.e., nitrate and nitrite) concentration was lower in HH than that in NH, suggesting altered systemic NO bioavailability by HH.

Nitrate and nitrite are physiologically converted back in blood and tissues to form NO, and remarkably, the nitrate–nitrite–NO pathway is gradually activated as oxygen tensions falls (26). Simultaneously, a decline of O_2 in arterial blood and respiratory cortical centers stimulates the carotid chemoreceptors and triggers AHVR (7). Interestingly, lower AHVR was observed in rats when NO production was inhibited (13). Similarly, Haxhiu et al. (16) found that NO synthase blockade attenuated the hypoxia-induced increase in respiratory activity in rats and that the increased respiratory output observed in AHVR resulted from the oxygen deprivation, leading to the activation of NO-cGMP-dependent pathway in the central nervous system.

Taken together, the results of these studies suggest that the lower minute ventilation in HH in our subjects could be due to a decrease in NO bioavailability. However, our results show an increased oxidative stress in HH compared with NH, supporting the speculation that exaggerated oxidative stress may both affect ventilation and NO bioavailability.

In the present study, NOx decreased in HH and remained stable in NH. This lowered NOx observed in HH could be explained by the increase in oxidative stress. Indeed, the increased oxidative stress revealed by higher plasma concentration of AOPP and SOD in HH might inhibit NO formation and reduce its bioavailability. This phenomenon of NO metabolism down-regulation associated with oxidative stress increase was observed recently (32). Mechanistically, reactive oxygen species (ROS) inducing oxidative stress impair the bioavailability of NO (39). More specifically, the superoxide anion ($O_2^{\cdot -}$) likely overproduced during hypoxia (14) can react with NO to form peroxynitrite ($ONOO^-$), thereby inducing NO inactivation and eNOS uncoupling. In this context, it was clearly shown that oxidative stress was increased during a similar normobaric hypoxic stimulus (12 h at a $P_{ET}O_2$ of 60 mm Hg) (31), strongly suggesting an overgeneration of ROS.

Interestingly, the plasma pH level was higher in NH compared with HH, and the higher SOD activity and levels of AOPP during HH suggest that ROS is likely more generated during HH by comparison with NH. This is in accordance with the enhanced free radical formation in case of a lower pH level (2,38). A relative acidosis can induce oxidative stress by causing protonation of the peroxynitrite anion (ONOO-), leading to radical hydroxyl (OH•) generation and by promoting delocalization of protein-bound iron stores, thereby accentuating redox stress induced by Fenton reaction (15,22). Actually, the increased ventilatory drive (as shown by the lower $P_{ET}CO_2$ during exercise) observed in NH likely decreases the blood H^+ concentration in line with our reported pH values. The proton being known as a

pro-oxidant particle, this respiratory alkalosis in NH may thus reduce the ROS production and explain the higher plasma oxidative stress measured in HH.

We also acknowledge that only half of the oxidative stress markers suggest higher ROS production. However, different oxidative pathways specific for each marker with respect to hypoxia exposure could explain these discrepancies. MDA was shown to be much less sensitive than other oxidative stress markers (24) because it represents the end product of the polyunsaturated fatty acid oxidation pathway. In the context of our slight physiological difference between HH and NH, it would be unlikely that MDA was able to detect such difference. Similarly, we already reported such observation (AOPP increase without MDA change) in athletes submitted to hypoxic training (vs identical normoxic training) (35). In addition, AOPP and MDA do not reflect the same oxidative stress pathways, that is, AOPP resulting from the myeloperoxidase pathway (44) via monocytes activation while MDA from polyunsaturated fatty acid oxidation. These results may suggest that hypoxia-induced oxidative stress could be more related to superoxide generation from NADPH oxidase activation than to an extracellular ROS production. In this present study, SOD was significantly higher at H + 24 but GPX was not affected during the 24 h of hypoxic exposure. SOD represents the first line of antioxidant enzyme in reducing the superoxides produced in the cell, whereas GPX share with catalase the reduction of H_2O_2. In addition, H_2O_2 is less reactive than superoxide. These antioxidant enzyme characteristics may suggest that SOD could be less stimulated than GPX for a similar superoxide production.

Finally, the lack of nitrotyrosine change despite higher oxidative stress in HH could result of the observed decreased NO bioavailability (demonstrated by lower NOx and exNO).

In conclusion, we strongly believe that the increase of AOPP and SOD may reflect a higher ROS overgeneration in HH compared with NH. The exercise-induced increase in ventilation throughout 24 h of hypoxic exposure was less in HH than that in NH and was associated with a decrease in NO bioavailability in HH. Furthermore, we observed increased oxidative stress and enzymatic antioxidants in HH. It can be hypothesized that the difference in oxidative stress and NO observed between HH and NH could explain the higher normobaric ventilatory responses to hypoxia. Although further investigation is needed to clarify how decreased NO bioavailability and increased oxidative stress could affect the lower ventilatory responses to hypoxia observed in HH, these new findings may have important potential consequences for adaptation and physical performance at altitude because different responses in ventilation and oxidative stress may influence benefits to athletes training or living at altitude for performance improvement (28).

It is still unclear if the reported differences in pH level, ventilatory responses, and nitrosative and oxidative stress between HH and NH have a clinical relevance (28). However, because it was recently shown that HH was more effective than NH as pre-acclimatization treatment to minimize AMS symptoms (11), we believe that further clinical investigations are worth of interest.

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