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Scientific Articles

Ventilatory Chemosensitivity, Cerebral and Muscle Oxygenation, and Total Hemoglobin Mass Before and After a 72-Day Mt. Everest Expedition

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Abstract

Cheung, Stephen S, Niina E. Mutanen, Heikki M. Karinen, Anne S. Koponen, Heikki Kyröläinen, Heikki O. Tikkanen, and Juha E. Peltonen. Ventilatory chemosensitivity, cerebral and muscle oxygenation, and total hemoglobin mass before and after a 72-day Mt. Everest expedition. *High Alt Med Biol* 15:331–340, 2014.—
Background: We investigated the effects of chronic hypobaric hypoxic acclimatization, performed over the course of a 72-day self-supported Everest expedition, on ventilatory chemosensitivity, arterial saturation, and tissue oxygenation adaptation along with total hemoglobin mass (tHb-mass) in nine experienced climbers (age 37 ± 6 years, $\dot{V}O_{2peak}$ $55 \pm 7\,mL \cdot kg^{-1} \cdot min^{-1}$).
Methods: Exercise-hypoxia tolerance was tested using a constant treadmill exercise of 5.5 $km \cdot h^{-1}$ at 3.8% grade (mimicking exertion at altitude) with 3-min steps of progressive normobaric poikilocapnic hypoxia. Breath-by-breath ventilatory responses, Spo_2, and cerebral (frontal cortex) and active muscle (vastus lateralis) oxygenation were measured throughout. Acute hypoxic ventilatory response (AHVR) was determined by linear regression slope of ventilation vs. Spo_2. PRE and POST (< 15 days) expedition, tHb-mass was measured using carbon monoxide-rebreathing.
Results: Post-expedition, exercise-hypoxia tolerance improved ($11:32 \pm 3:57$ to $16:30 \pm 2:09$ min, $p < 0.01$). AHVR was elevated (1.25 ± 0.33 to 1.63 ± 0.38 $L \cdot min^{-1} \cdot \%^{-1}$ Spo_2, $p < 0.05$). Spo_2 decreased throughout exercise-hypoxia in both trials, but was preserved at higher values at 4800 m post-expedition. Cerebral oxygenation decreased progressively with increasing exercise-hypoxia in both trials, with a lower level of deoxyhemoglobin POST at 2400, 3500 and 4800 m. Muscle oxygenation also decreased throughout exercise-hypoxia, with similar patterns PRE and POST. No relationship was observed between the slope of AHVR and cerebral or muscle oxygenation either PRE or POST. Absolute tHb-mass response exhibited great individual variation with a nonsignificant 5.4% increasing trend post-expedition (975 ± 154 g PRE and 1025 ± 124 g POST, $p = 0.17$).
Conclusions: We conclude that adaptation to chronic hypoxia during a climbing expedition to Mt. Everest will increase hypoxic tolerance, AHVR, and cerebral but not muscle oxygenation, as measured during simulated acute hypoxia at sea level. However, tHb-mass did not increase significantly and improvement in cerebral oxygenation was not associated with the change in AHVR.

Key Words: acute hypoxic ventilatory response; altitude adaptation; chronic hypoxia; climbers; extreme altitude; NIRS.

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Introduction

Acute exposure to altitude significantly decreases endurance exercise performance, but this decrement may be reversed with chronic exposure and acclimatization through a wide range of physiological mechanisms (Moore et al., 1998; Fulco et al., 2013). Ventilation rapidly increases upon acute hypoxic exposure, and this hypoxic ventilatory response (HVR) is generally presumed to enable better performance at altitude by maintaining arterial oxygen saturation levels (Schoene et al., 1984; Masuyama et al., 1986). Alternatively, a strong HVR has also been proposed to be a negative adaptation that reduces ventilatory reserve at altitude (Bernardi et al., 2006), while hyperventilation and the resultant hypocapnia may reduce cerebral blood flow (Poulin et al., 2002; Ainslie and Poulin, 2004).

Cerebral oxygenation does not appear to be a critical threshold limiter for self-paced (Billaut et al., 2010) or maximal exercise capacity (Subudhi et al., 2007) in normoxia. However, when combined with the additional stress of acute or chronic exposure to hypoxia, cerebral oxygenation rose and exercise tolerance was prolonged when hyperoxia was induced near the point of exhaustion (Subudhi et al., 2008), suggesting that oxygen availability in the brain may become critical limiters to performance in hypoxia. Comparing the responses to isocapnic hypoxia with normoxia while resting (Peltonen et al., 2007) or to poikilocapnic hypoxia during graded exercise to exhaustion (Peltonen et al., 2009), cerebral oxygenation tended to decrease at a greater rate during hypoxia, although no single threshold concurrent with voluntary fatigue was apparent; muscle oxygenation levels decreased at a similar rate in both normoxia and hypoxia. Similar observations were reported by Ainslie et al. (2008), with a decrease in cerebral oxygenation but less change in muscle oxygenation during hypoxic exercise following either 10–12 days of intermittent hypoxia or chronic exposure to 1500 m elevation. In addition, arterial oxygen saturation levels may serve as a predictor for acute mountain sickness (Karinen et al., 2010). Fulco et al. (2011) reported improved arterial oxygen saturation levels during sleep to be reflected in improvements in AMS symptoms upon awakening but not on AMS or exercise performance for the remainder of the day. It would be of interest to explore whether chronic altitude exposure alters arterial oxygen saturation levels during exercise, along with its relationship to tissue oxygenation.

Another commonly proposed adaptation with chronic hypoxic exposure is an elevation in the release of erythropoietin (EPO) (Pugh, 1964), potentially stimulating the production of red blood cells and ultimately enabling greater oxygen delivery to the body for a given cardiac output (Gunga et al., 2007). Pugh (1964) reported an increase in [EPO] and hemoglobin concentration [Hb] with prolonged expeditions above 5500 m. Similarly, Savourey et al. (1996) reported an increase in [EPO] in nine climbers following a 62-day Himalayan expedition, which persisted for up to a month upon return to sea level. However, [EPO] and [Hb] are highly influenced by plasma and blood volume, which can alter rapidly and transiently upon ascent or return to sea level (Tannheimer et al., 2010).

In contrast, total hemoglobin mass (tHb-mass) is the actual endpoint hematological result of hypoxic exposure. With prolonged moderate altitude (2300–3000 m) exposure a progressive increase of approximately 1.1% in tHb-mass for each 100 h of hypoxic exposure over 2 weeks of moderate altitude has been reported in a meta-analysis (Gore et al., 2013), with a proposed ceiling increase across multiple studies of approximately 7% after ~500 h (Saunders et al., 2009). The actual measurement of tHb-mass before and following self-supported high-altitude climbing expeditions has not been previously reported. Furthermore, wide inter-individual variability in hematological responses to moderate altitude exposure has also been reported, with distinct responders and nonresponders in [EPO], red cell volume, and aerobic capacity following 30 h of live-high-train-low altitude exposure (Chapman et al., 1998); similarly, highly variable tHb-mass responses also occur in response to live-high-train-high altitude exposures (Saunders et al., 2009b). The variability in tHb-mass responses to extreme altitude has not been investigated.

The aim of the present study was to examine the physiological responses to an extreme high-altitude mountaineering expedition, focusing on the integrative adaptations in ventilatory responses, arterial saturation, cerebral and active muscle oxygenation, and total hemoglobin mass. Before and following a 72-day self-supported Everest mountaineering expedition, a constant treadmill exercise protocol with an incremental poikilocapnic hypoxic challenge was performed, along with measurement of total hemoglobin mass. We hypothesized that exercise-hypoxia tolerance would be improved post-expedition. Furthermore, we hypothesized the following to be contributing mechanisms:

1. Ventilatory chemosensitivity would be enhanced, with an elevated acute hypoxic ventilatory response post-expedition.
2. Arterial O_2 saturation would be higher post-expedition at a given simulated altitude due to increased acute hypoxic ventilatory response.
3. Cerebral and active muscle (vastus lateralis) oxygenation would be maintained at a higher level post-expedition due to increased hypoxic ventilatory response.
4. Total hemoglobin mass would be increased post-expedition.

Materials and Methods

Participants

This study was approved by the institutional research board and the University of Helsinki ethics committee, and it was conducted according to the Declaration of Helsinki. Nine healthy males (height 179 ± 7 cm, body mass 80.4 ± 8.6 kg, age 37 ± 6 years, body mass index 25.1 ± 2.6, $\dot{V}O_{2peak}$ 55 ± 7 mL·kg^{-1}·min^{-1}) took part in the study after providing informed written consent. The participants were experienced climbers and members of the Airborne Ranger Club of Finland taking part in an expedition to summit Mount Everest. Medical screening by a physician, including standard 12-lead ECG at rest and flow-volume spirometry (Medikro Spiro 2000, Medikro, Kuopio, Finland), was performed before the start of the study. None of the participants had a history of cardiovascular, respiratory, or musculoskeletal diseases, and all were free of medication.

Climbing Expedition

Expedition day 1 was defined as the day of arrival in Kathmandu. At the beginning and at the end of the expedition,

the climbers spent 5 and 6 days, respectively, in Kathmandu at an altitude of 1300 m. The climbers first hiked to the base camp of Mount Everest (5300 m) and reached progressively higher camps at various altitudes. Four out of nine climbers reached the Everest summit (8848 m), all with oxygen supplementation beginning at approximately 8100 m, after 59 days. Each climber reached at least the altitude of 7100 m. For all participants, the expedition lasted 72 days in total, including a 2-month stay at an altitude above 2500 m from which 49 days were at or above 5300 m. The expedition was self-supported, in that no porters were used above 5300 m and the nine climbers carried all of their own gear. Thus, overall activity levels and energy expenditure were much higher than typical supported mountaineering expeditions. For example, each climber performed 9–12 traverses of the Khumbu Icefall compared to the typical 2–3 traverses for a supported expedition. In general, all climbers experienced mild acute mountain sickness (3–4 on the Lake Louise Scale) upon initial arrival at 5300 m, but these symptoms faded and did not recur at higher altitudes.

Exercise-hypoxia protocol

The experimental protocol was performed twice with each participant, on average 12 days before (PRE, range 6–20 days) and 13 days (POST, range 9–15 days) after the expedition descended to 3000 m. Measurements were performed at sea level (Helsinki) at ambient temperature and humidity for both pre- and post-tests. For both PRE and POST, the participants arrived at the laboratory 3–4 hours after a meal and after 24 h without caffeine or alcohol ingestion, along with no physical exercise for at least 12 h.

The protocol consisted of walking on a treadmill with a constant load (speed 5.5 $km \cdot h^{-1}$, grade 3.8%) while being exposed to 3-min stages of increasing hypoxic stimulus (P_{IO_2} = 159, 120, 105, 90, 86, 80, 75, 69 mmHg) to simulate the different camp altitudes during an ascent to Mt. Everest (0, 2400, 3500, 4800, 5200, 5800, 6400, 7100 m, respectively). The actual hypoxic exposure was preceded by 3 min of rest while the participant stood relaxed and breathed normoxic room air through a mask. The participant then began a 3 min baseline walking in normoxia, after which hypoxic breathing was started. The duration of each hypoxic step (3 min) was chosen to ensure enough time for the adjustment of gas mixture and for the measured variables to settle to a new level of P_{IO_2}, based on 6 x the time constant (τ) for rate of oxygen uptake ($\dot{V}O_2$) and heart rate of $\sim$30 s. At the same time, a total hypoxic duration < 20 min was chosen to minimize possible hypoxic ventilatory decline during longer exposures (Kolb et al., 2004).

The normobaric, poikilocapnic, hypoxic protocol was achieved with a custom-designed flow meter. The hypoxic stimulus was varied by reducing the inspired O_2 concentration, while the total pressure of gas mixture was kept normobaric by simultaneously increasing N_2 concentration. The apparatus consisted of gas bottles (O_2 and N_2), adjustment device for O_2 and N_2 gas flow, mixing chamber for inspiratory gases and a tube to conduct gases from the mixing chamber to valve. A three-way valve directed either normoxic room air or O_2/N_2 mixtures to the inspiratory port of a non-rebreathing valve. The adjustments of inspiratory O_2 and N_2 gas flow were made by comparing the measured fractional gas concentration of inspired O_2 and N_2 with the desired values and adjusting the gas mixture manually as needed. To ensure participant well-being, exercise was immediately terminated upon voluntary decision, if the participant started to exhibit impaired coordination of walking, or if arterial O_2 saturation (Spo_2) fell to an ethically-imposed limit of 62%. Immediately after the termination of the test, the participants were given 100% O_2 via respiratory mask to speed up the recovery of Spo_2 to the normal level.

Cardiorespiratory measures

Spo_2 was measured by pulse oximetry (Nonin 9600, Nonin Medical, Inc., Plymouth, USA) on the right forefinger tip. Ventilation ($\dot{V}_E$) and alveolar gas exchange including end-tidal partial pressures for O_2 and CO_2 ($PETO_2$ and $PETCO_2$, respectively) were measured breath-by-breath throughout the test. The inspiratory and expiratory flow and volumes were monitored by a low-deadspace, low-resistance turbine (Triple V, Jaeger Mijnhardt, Bunnik, The Netherlands) connected to a mask (Hans Rudolph Inc., Kansas City, MO). The turbine was calibrated before each test by a 3.00-L syringe (Hans Rudolph Inc.). Inspired and expired gases were sampled continuously at the mouth and the concentrations of O_2, CO_2, N_2, and argon were analyzed by mass spectrometry (AMIS 2000, Innovision, Odense, Denmark) after calibration with precision analyzed gas mixtures. The raw data were transferred to a computer where gas delays were determined for each breath to align concentrations with volume data, and to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated with the AMIS algorithms, which are slightly modified from the original algorithms of Beaver et al. (1981) and interpolated to give second by second values. In this study $PETCO_2$ was used as an index of arterial CO_2 pressure instead of direct measurement.

For both pre- and post-expedition testing, acute hypoxic ventilatory chemosensitivity (AHVR) was obtained by calculating the linear regression slope of $\dot{V}_E$ versus Spo_2 ($L \cdot min^{-1} \cdot \%^{-1}$) from the beginning of normoxic exercise up to a standardized hypoxic load (4800 m above sea level) and also to the peak hypoxic load achieved by each participant (normoxic exercise – peak hypoxic exercise). Thus, ventilation was plotted as a function of Spo_2 on a second-by-second basis and the slope of the regression line, $\Delta\dot{V}_E/\Delta SpO_2$, was defined as AHVR during standard walking but increasing hypoxic challenge. This method to determine AHVR during exercise has been used previously by Sato et al. (1996) and Peltonen et al. (2009).

Near-infrared spectroscopy

Regional cerebral (frontal cortex or FC) and muscle (vastus lateralis or VL) tissue oxygenation profiles were monitored noninvasively by near-infrared-spectroscopy (NIRS) (Oxymon, Artinis Medical Systems, Zetten, the Netherlands), which enabled continuous monitoring of tissue saturation index (TSI) along with relative concentration changes in oxy- ($\Delta[O_2Hb]$), deoxy- ($\Delta[HHb]$), and total ($\Delta[tHb]$) hemoglobin. For both cerebral and muscle measurements, the optodes with three transmitters, operating at wavelengths of 765 and 860 nm, and one receiver were housed in an optically dense plastic holder and attached firmly on the skin using two-sided and one-sided tape. The FC optode was additionally fixed with a headband. The cerebral probe was located over the right frontal cortex, on

average ~3 cm above the right eyebrow and ~4.5 cm lateral from the middle line of the forehead. This site over the prefrontal cortex is assumed to be involved in the higher aspects of motor control and the planning of voluntary movement (Sahyoun et al., 2004). The vastus lateralis was chosen to represent active muscle, as it is a powerful knee-extensor that is activated at several phases of the walking gait cycle (Murray et al., 1984). Muscle optodes were placed on the right vastus lateralis in parallel with the long axis of the muscle, ~14 cm above the upper edge of the patella and ~4 cm lateral from the middle line. The inter-optode distances were 40 mm for vastus lateralis and 45 mm for frontal cortex but they were changed, if needed, when checking the quality of signals before starting the measurements. Before fixing optodes hair, if existed, was shaved and the skin was wiped. The exact place of all optodes was marked and measured individually during both tests. Adipose tissue thickness from the thigh was measured with skinfold caliper PRE and POST to control effect of subcutaneous adipose tissue on the signal.

The theory of NIRS and details on its use in exercise have been described in detail elsewhere (Boushel et al., 2001). Briefly, the intensity of incident and transmitted light was recorded continuously and, along with the specific extinction coefficients and optical path length, used for on-line estimation and display of concentration changes ($\Delta\mu$M) from the resting baseline of $\Delta[O_2Hb]$, Δ[HHb], and total hemoglobin Δ[tHb]. The values used for the differential pathlength factor (DPF) were 5.51 for the leg (van der Zee et al., 1992; Duncan et al., 1995), and calculated for cerebral tissue as $DPF = 4.99 + 0.067 \times Age^{0.814}$ according to the manufacturer's guidelines. The tissue saturation index (TSI, % = $[O_2Hb]$/[tHb]) was calculated from the light attenuation slope along the distance from the three emitting points as detected by the sensor in the receiving optode. Data from NIRS were averaged to give values in 1 sec intervals, and time-aligned with gas exchange, heart rate, and Spo_2 data. When performing NIRS analyses, the values obtained during exercise-hypoxia were compared with the values of normoxic walking exercise instead of rest because, at the onset of exercise, muscle pumping expels blood from the muscles towards the heart, which is expected to explain the rapid temporary changes on NIRS measurements (DeLorey et al., 2003).

Total hemoglobin mass measurement

During a visit to the laboratory either a day before or after the exercise-hypoxia test, total hemoglobin mass was assessed using CO rebreathing (COR) as described in detail by Schmidt and Prommer (2005) and modified by Prommer and Schmidt (2007). In brief, after 15-min resting in a sitting position, the subjects were connected to a specially designed closed spirometric system allowing a CO-bolus application, followed by 2 min rebreathing of approximately 3 L amount of oxygen. The administered amount of CO in mL was individually calculated (mL CO = 1.0 x body weight (kg)). During COR, the whole apparatus was checked for CO leakage with a portable CO-gas analyzer (Dräger Pac 7000, Dräger Safety AG & Co. KGaA, Lübeck, Germany). This analyzer was also used to calculate the amount of CO that had not been taken up by the body from the remaining CO in the spirometer and the residual volume of the lung, as well as from the CO exhaled after disconnecting the subject from the spirometer, at min 4 after the beginning of the COR period.

Blood samples were drawn from a fingertip immediately before, 6 and 8 min after the start of the COR period. For %HbCO and hemoglobin concentration measurements, blood was drawn into two 100 μL preheparinized capillaries (Clinitubes, Radiometer Medical ApS, Copenhagen, Denmark). After the blood drawing, the tubes were closed and mixed with a wire and magnet. Each of the two samples was measured immediately in triplicate by oximetry (OSM3, Radiometer, Copenhagen, Denmark) and the average of the replicates was used for further calculations, as suggested by Alexander et al. (2011) to minimize analyzer error to $<1\%$. Daily, the OSM3 was calibrated to standard and the quality of measurement was checked by means of three different quality control solutions. tHb-mass, erythrocyte volume, plasma volume and blood volume were calculated by the SpiCO Software 1.0 (Blood tec, Bayreuth, Germany), which was developed and patented by the founders Schmidt and Prommer (2005), and Prommer and Schmidt (2007). The tHb-mass was calculated based on the formula: tHb mass (g) = K x MCO x 100 x $((\Delta\%HbCO \times 1.39)^{-1}$, where K is ambient barometric pressure (mmHg) x 760^{-1} (mmHg) x [1 + (0.003661 x ambient temperature °K)], MCO is the volume of CO (mL) applied to the system minus the sum of CO volume remaining in the spirometer and the volume that was exhaled from minute 2–8 (the CO volume not bound to hemoglobin), Δ%HbCO is the difference between the initial %HbCO (mean %HbCO of two initial blood samples) and maximal %HbCO (average %HbCO of blood samples at minutes 6 and 8 after starting the COR), and 1.39 (mL g^{-1}) is Hüfners number as 1.39 mL CO bind to 1 g Hb.

The software program SpiCO corrects for the loss of CO from hemoglobin to myoglobin according to the findings of Schmidt and Prommer (2005) and by Prommer and Schmidt (2007) by 0.3% of administered CO per minute. The calculation of EV = (644 x Hct (%) x $[Hb]^{-1}$ (g/L)) x tHb-mass (mmol) is based on the equations 1–3 presented by Burge and Skinner (1995). The typical error (%TE) of the tHb-mass measurement in our laboratory obtained by duplicate tests is 1.7% (95% confidence limit 1.3%–2.4%) for relative tHb-mass and 2.0% (95% CL 1.5%-2.8%) for absolute tHb-mass (Koponen et al., 2013). This is similar to Schmidt and Prommer (2005) who reported the reliability of the tHb-mass measurement as 1.7% TE (95% CL 3.3%).

Statistical analyses

Exercise-hypoxia tolerance, thigh skinfold thickness, hematological responses (tHb-mass; erythrocyte, plasma, and blood volumes), and AHVR (from 0–4800 m and from 0 m, peak hypoxia) were compared pre/post-expedition using a paired *t*-test. For the exercise-hypoxia test, the mean values of the last 30 sec of each exercise-hypoxic step were chosen for statistical analysis. Cardiorespiratory variables ($\dot{V}_E$, heart rate), arterial oxygen saturation, along with cerebral and muscle oxygenation variables over the course of exercise-hypoxia were compared using a one-way repeated measures ANOVA with Holm-Sidak post-hoc analysis. Comparisons between PRE and POST at a given altitude and at peak altitude was performed by paired *t*-test. The simulated altitudes of 0–4800 m were used as the common levels of comparison, as 4800 m represented the highest hypoxic level tolerated by all participants both PRE and POST. Relationships between cerebral and vastus lateralis TSI versus AHVR were tested by

Pearson product correlation. All data were checked for normal distribution and statistical analyses were performed using PASW Statistics 18.0 software (SPSS Inc., Chicago, IL, USA). Results are expressed as means ± SD unless otherwise indicated. A p value of <0.05 was considered statistically significant.

Results

Hypoxic exercise and ventilatory response

During PRE, all participants completed 3 min of walking at 3500 m and were able to exercise at 4800 m for at least 1 min. Hypoxic-exercise tolerance improved in POST, with all participants completing exercise at 5200 m and able to walk at 5800 m for at least 1.5 minutes. The progressive hypoxia was tolerated for 11:32 ± 3:57 (min:sec) in PRE and 16:30 ± 2:09 in POST ($p<0.01$), with increased exercise time in 8 of 9 participants. Ventilatory and arterial saturation data are presented in Figure 1. Ventilation during both PRE (df = 4, F = 33.3, $p<0.001$) and POST (df = 6, F = 39.6, $p<0.001$) exercise-hypoxic exposure progressively increased throughout each exercise-hypoxic stage from 0 m to peak hypoxic stage. Between 0 m to 4800 m, $\dot{V}_E$ was higher POST ($p<0.05$) at 3500 m. Compared to 0 m exercise, $PETCO_2$ was lower during PRE (df = 4, F = 39.3, $p<0.001$) from 3500 m through to peak hypoxic exposure and in POST (df = 6, F = 142.0, $p<0.001$) at all exercise-hypoxic stages. $PETCO_2$ was lower during POST than PRE at rest, 2400 m, and 3500 m. Spo_2 steadily decreased over the course of each exercise-hypoxic stage in both PRE (df = 4, F = 93.6, $p<0.001$) and POST (df = 6, F = 104.0, $p<0.001$) from 0 m exercise through to peak exercise-hypoxic stage; between PRE and POST, Spo_2 were similar up to 3500 m, but was maintained at a higher level at 4800 m ($p<0.001$) during POST. Spo_2 at peak exposure were similar, at 65 ± 4 and 64 ± 3% for peak exercise-hypoxia in PRE and POST, respectively, but in POST this level was achieved at greater simulated altitude. Acute hypoxic ventilatory response, defined as the increase in $\dot{V}_E$ per percentage decrease in arterial saturation (Spo_2) from 0 m exercise to 4800 m hypoxia, increased sharply following chronic hypoxia in 7 of 9 participants, with 1 unchanged and 1 decreasing (Table 1).

Cerebral and muscle oxygenation

As compared to the normoxic walking, cerebral oxygenation was reduced over the course of each exercise-hypoxic stage in both the PRE and POST trials (Fig. 2), as indicated by progressively decreasing TSI (PRE: df = 4, F = 86.5, $p<0.001$; POST: df = 6, F = 65.3, $p<0.001$) and $\Delta[O_2Hb]$ (PRE: df = 4, F = 69.8, $p<0.001$; POST: df = 6, F = 104.6, $p<0.001$), and increasing Δ[HHb] (PRE: df = 4, F = 57.5, $p<0.001$; POST: df = 6, F = 68.5, $p<0.001$). Comparing between PRE and POST trials, the levels of Δ[HHb] were significantly lower during POST at 2400, 3500, and 4800 m ($p<0.05$ to 0.01).

Tissue saturation index at the vastus lateralis were similar to 0 m exercise at both 2400 and 3500 m, but decreased from 4800 m and beyond in both PRE (df = 4, F = 23.0, $p<0.001$) and POST (df = 6, F = 43.4, $p<0.001$) testing (Fig. 3). In POST compared to PRE, TSI was similar up to 4800 m, but was significantly lower during peak exercise-hypoxia ($p<0.05$); the TSI slope (i.e., the rate of deoxygenation from 0 m to peak hypoxia) was significantly greater in POST than in PRE (−0.63 and −0.46 $\%\cdot\%^{-1}$, respectively). Leg Δ[HHb] (PRE: df = 4, F = 23.3, $p<0.001$; POST: df = 6, F = 67.8, $p<0.001$), and Δ[tHb] (PRE: df = 4, F = 25.5, $p<0.001$; POST: df = 6, F = 130.6, $p<0.001$) increased in both tests, with no differences in Δ[HHb] or in Δ[tHb] between PRE and POST up to 4800 m or at peak exercise-hypoxia. The Δ[HHb] slope was greater in POST than in PRE (0.429 and 0.279 $\mu M\cdot\%^{-1}$, respectively, $p<0.05$). In PRE, $\Delta[O_2Hb]$ remained at normoxic walking levels during first two hypoxic steps and started to decrease at 4800 m (df = 4, F = 6.5, $p<0.001$). In POST, $\Delta[O_2Hb]$ was initially steady, then decreased to significantly lower level than at 0 m exercise at 5800 m and peak exercise-hypoxia (df = 6, F = 16.4, $p<0.001$). There were no differences in leg $\Delta[O_2Hb]$

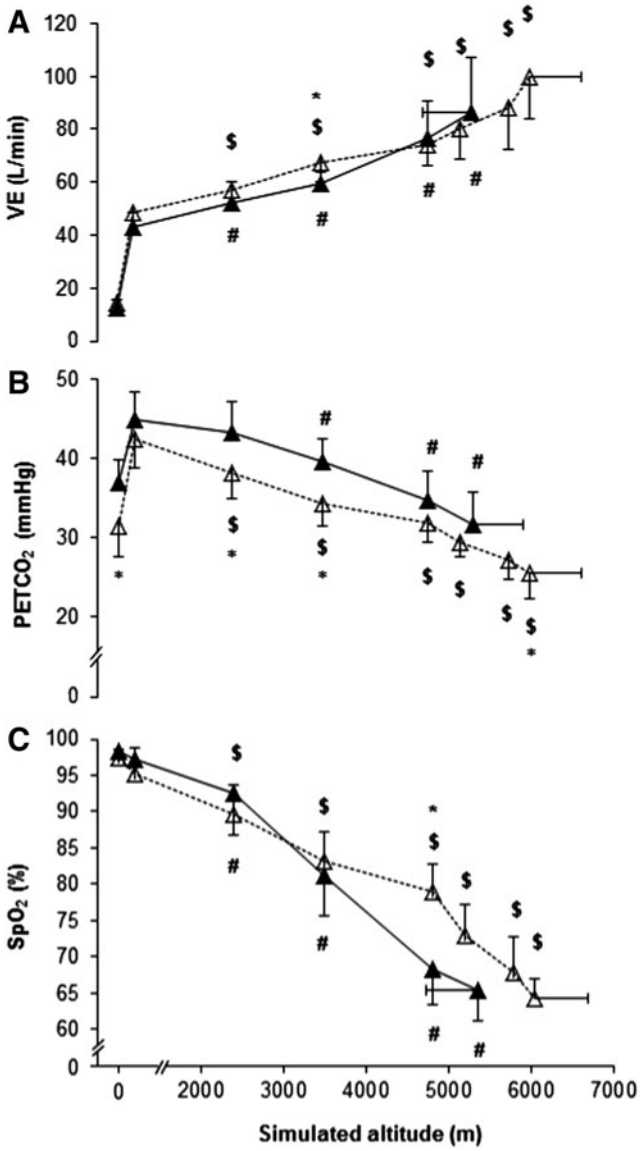

FIG. 1. **(A)** Ventilation; **(B)** end-tidal CO_2; and **(C)** arterial oxygen saturation responses to exercise-hypoxia during PRE (▲) and POST (△). *Significantly different from PRE ($p<0.05$). #Significantly different from normoxic walking in PRE ($p<0.05$). $Significantly different from normoxic walking in POST ($p<0.05$). In order to improve the clarity of the figure, the x-value of baseline walking is artificially raised to 200 m.

Table 1. Body Mass, Total Hemoglobin Mass, Erythrocyte Volume, and Ventilatory Chemosensitivity Responses Pre- and Post-Expedition

	Body mass (kg)		*Plasma volume (mL)*		*Total blood volume (mL)*		*[Hb] ($g \cdot dL^{-1}$)*		*tHb-mass (g)*		*EV (mL)*		*AHVR (0 - 4800 m) ($L \cdot min^{-1} \cdot \%^{-1}$)*	
	PRE	*POST*	*PRE*	*POST*	*PRE*	*POST*	*PRE*	*POST*	*PRE*	*POST*	*PRE*	*POST*	*PRE*	*POST*
1	81.3	79.6	4879	4908	8294	8132	14.5	14.5	1095	1074	3415	3224	1.31	1.78
2	86.7	83.3	5743	4786	9454	8083	14.4	15.1	1241	1113	3712	3298	1.11	2.35
3	68.7	67.3	3422	2719	5585	5252	13.9	17.0	706	811	2163	2533	1.00	1.03
4	71.5	71.6	4369	3929	7147	6859	14.2	15.2	927	949	2779	2929	0.90	1.26
5	70.1	68.4	3668	3853	6234	6699	14.3	14.7	813	895	2567	2846	1.69	1.83
6	85.0	80.9	4925	4710	8213	8188	13.7	14.9	1025	1107	3287	3479	0.83	1.66
7	88.2	84.0	4562	3983	7559	7182	14.4	16.0	992	1046	2997	3200	1.17	1.60
8	97.2	88.8	4576	4199	7297	8028	14.9	16.7	987	1220	2921	3829	1.73	1.78
9	80.2	78.6	4600	4229	7574	7284	14.4	15.3	994	1014	2974	3055	1.49	1.41
Mean	81.0	78.1	4505	4146	7484	7301	14.3	15.5	975	1025	2979	3155	1.25	1.63
SD	9.5	7.4	686	663	1140	961	0.4	0.9	154	124	461	376	0.33	0.38
P value	0.01*		0.02*		0.38		0.01*		0.17		0.19		0.03*	

AHVR, acute hyperventilatory response; EV, erythrocyte volume; [Hb], hemoglobin concentration; tHb-mass, total hemoglobin mass. *Significantly different PRE to POST.

between the PRE and POST up to 4800 m or at peak exercise-hypoxia. No significant relationship was evident in either the cerebral or vastus lateralis sites between the magnitude of the AHVR and the slope for TSI with decreasing Spo_2 during either PRE or POST.

Red blood cell responses

The expedition elicited no significant change in total blood volume along with a plasma volume decrease post-expedition (Table 1). Hemoglobin concentration significantly increased post-expedition (14.3 ± 0.4 PRE to $15.5 \pm 0.9\ g \cdot dL^{-1}$ POST, $p = 0.01$), but absolute total hemoglobin masses were similar ($p = 0.17$) at 975 ± 154 g (PRE) and 1025 ± 124 g (POST). A large inter-individual variability existed in the direction and magnitude of the tHb-mass changes, ranging from -14.5% to 20.2%, with seven participants increasing and two decreasing post-expedition. Erythrocyte volume also demonstrated a similar pattern of no overall changes post-expedition ($p = 0.19$) and wide inter-individual variability, ranging from -10.3% to 31%.

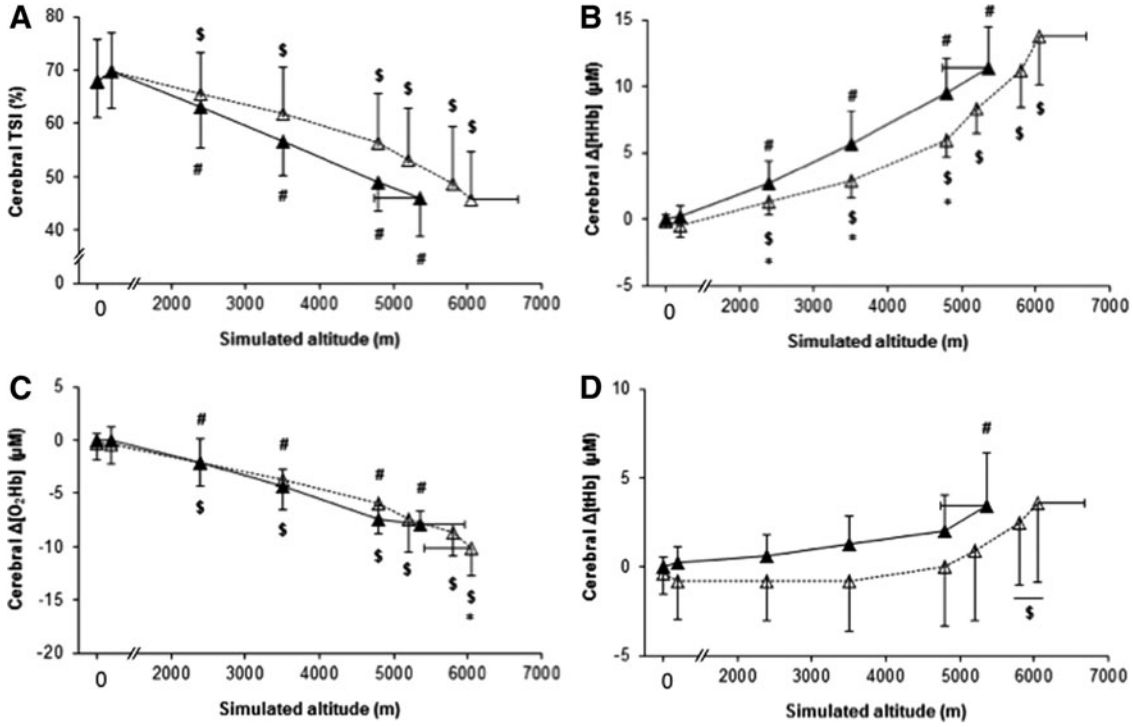

FIG. 2. Frontal cortex oxygenation responses to exercise-hypoxia during PRE (▲) and POST (△). *Significantly different from PRE ($p < 0.05$). #Significantly different from normoxic walking in PRE ($p < 0.05$). $Significantly different from normoxic walking in POST ($p < 0.05$). In order to improve the clarity of the figure, the x-value of baseline walking is artificially raised to 200 m.

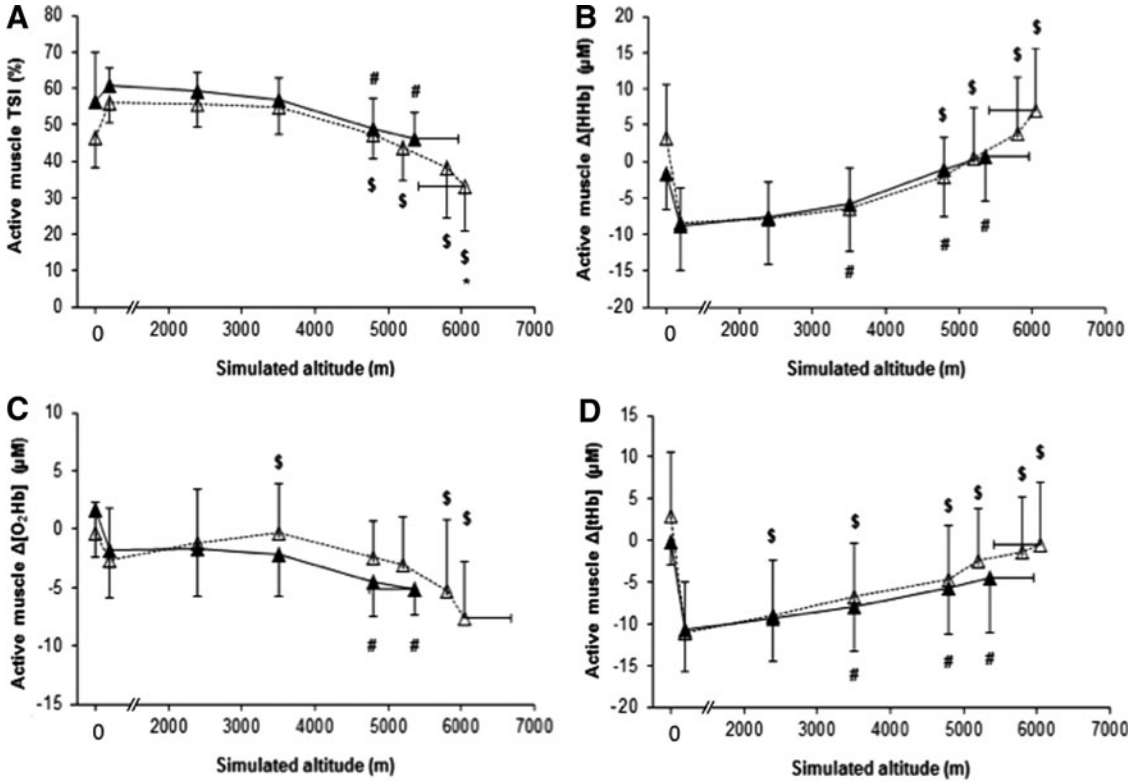

FIG. 3. Vastus lateralis oxygenation responses to exercise-hypoxia during PRE (▲) and POST (△). *Significantly different from PRE ($p < 0.05$). #Significantly different from normoxic walking in PRE ($p < 0.05$). $Significantly different from normoxic walking in POST ($p < 0.05$). In order to improve the clarity of the figure, the x-value of baseline walking is artificially raised to 200 m.

Discussion

To our knowledge, this is the first study on the effects of an extended mountaineering expedition on the combined ventilatory, arterial saturation, cerebral and muscle oxygenation, and total hemoglobin mass responses. We utilized a constant exercise with increasing levels of poikilocapnic hypoxia and investigated the potential and mechanisms for adaptation by performing the test before and following a self-supported Mount Everest expedition. A greater tolerance to an acute exercise-hypoxia challenge existed post-expedition, and acute hypoxic ventilatory response was enhanced while arterial oxygen saturation was maintained at higher levels post-expedition. Cerebral oxygenation was better maintained post-expedition, yet the degree of AHVR either before or following the expedition was not associated with either cerebral or active muscle oxygenation. Total hemoglobin mass tended towards increasing post-expedition, but high inter-individual variability precluded any group significance.

Hyperventilation is a common response to acute and chronic hypoxia, and our data demonstrated increasing $\dot{V}_E$ over the acute exercise-hypoxia challenge both pre- and post-expedition (Savourey et al., 1996). As our protocol maintained a constant exercise with step increases in hypoxia, this supports the idea that the hyperventilation was driven by the poikilocapnic hypoxia stimulus itself rather than any exercise-induced changes in metabolic demands or afferent (e.g., mechanoreceptor) feedback seen in prior studies utilizing a single hypoxic stage with a graded exercise test (Subudhi et al., 2007; Peltonen et al., 2009). In general, hyperventilation drives down $PETCO_2$ and leads to a higher pH and an increased hemoglobin binding affinity for oxygen, thus potentially helping to preserve arterial saturation overall. The acute hypoxic ventilatory response, calculated as the increase in $\dot{V}_E$ for a set decrease in Spo_2 from 0–4,800 m (Table 1), was increased by 30% post-expedition. This suggests that ventilatory chemosensitivity was enhanced over the expedition, possibly contributing to the maintenance of arterial saturation. A protective effect in Spo_2 was evident at 4800 m and beyond, with a rightward shift of approximately 1000 m for an equivalent arterial oxygen saturation as during pre-expedition (Fig. 1). While lower Spo_2 values of 60% or less have been reported in the field during moderate exercise after acclimatization at 5800 m (West et al., 1962), more recent field data on expedition climbers exercising at 5300 m reported Spo_2 values > 70% (Karinen et al., 2010), similar to our current data. Overall, our data suggest that chronic hypoxic acclimatization enhanced ventilatory chemosensitivity, but that these adaptations only became expressed in preserving higher arterial saturation once the body reached a moderate threshold of exercise and/or hypoxic stress.

The actual level of oxygen availability at the brain or muscle tissues may be altered by altitude acclimatization. The possible effects of chronic hypoxia on cerebral blood flow (Ainslie et al., 2007; Lucas et al., 2010) and subsequently on cerebral oxygenation, were beyond the scope of the present study. Cerebral oxygenation in our study, as measured using NIRS, changed similarly during exercise-hypoxia both pre- and post-expedition, with progressively decreasing TSI and $\Delta[O_2Hb]$ along with an increasing

Δ[HHb]. No significant differences occurred between pre- and post-expedition trials in TSI or in Δ[O_2Hb], though a trend towards improved cerebral TSI existed in POST. Vastus lateralis skinfold thickness did not differ significantly PRE and POST (8.1 ± 2.6 and 5.8 ± 3.2 mm, $p = 0.09$). This lack of difference in skinfold thickness, along with the NIRS data being similar PRE/POST at the 0 m exercise, supports the idea that any changes in NIRS during exercise-hypoxia probably reflect acclimatization rather than skinfold thickness differences. Similar to cerebral values, muscle oxygenation pre-expedition gradually decreased over the course of exercise-hypoxia, with progressive decreases in TSI and increasing Δ[HHb] as the level of hypoxia increased. The expedition did not appear to impact muscle oxygenation, as muscle oxygenation continued to decrease with further levels of hypoxia in both PRE and POST, with similar values and patterns for TSI, Δ[O_2Hb], Δ[HHb], and Δ[tHb] up to a simulated 4800 m. Therefore, any differences post-expedition appear primarily due to the greater level of exercise-hypoxia during POST rather than any specific acclimatization changes to muscle oxygenation.

A strong HVR has been proposed as a predictor for altitude performance (Schoene et al., 1984), with reports of higher HVR in elite climbers compared to sedentary controls and endurance athletes (Schoene, 1982). However, the actual mechanism for any HVR protection remains unknown, and some argue that a strong HVR may form a negative response due to diminishing ventilatory reserve (Bernardi et al., 2006). Ainslie and Poulin (2004) reported that, across individuals, a higher AHVR during acute poikilocapnic hypoxia was associated with a decrease in the acute hypoxic cerebral blood flow response, but propose that this may be due to the hyperventilation already inducing a hypocapnic-induced cerebral vasoconstriction and dampening the possibility for further responsiveness. In our study, despite a wide inter-individual variability in AHVR both before and following the expedition, no significant relationship existed between AHVR and the slope for cerebral or vastus lateralis TSI changes, suggesting that neither a higher nor lower ventilatory response to hypoxia impacted the actual oxygenation at the brain or active musculature.

Absolute total hemoglobin mass exhibited a nonsignificant trend towards increasing post-expedition, but the wide range of responses may have dampened overall group effects and highlights the wide inter-individual variability in response to altitude. Seven climbers increased their tHb-mass (range 2.0%–23.7%), but a reduction (1.9%–10.3%) was seen in two climbers. These changes are greater than the CV of our COR method, suggesting that these results are most likely real and not a measurement error. Also, our testing window of 9–15 days post-expedition was similar to the 14 days reported by Prommer et al. (2010) for maintenance of elevated tHb-mass upon descent to sea level.

Several possibilities may explain the lack of tHb-mass elevation or its wide variability. The expedition being self-supported would have increased the overall physical stress, and this may have affected the ability to produce red cells and hemoglobin synthesis. Our data are also consistent with equivocal findings of hematological responses to chronic moderate altitude exposure, with both 'responders' and 'non-responder' in [EPO] and running performance following a 'live-high-train-low' altitude training regimen (Chapman et al., 1998)—with only the 'responders' exhibiting an increased total red cell volume. Variability is also evident when actually measuring total hemoglobin mass at moderate altitude. Siebenmann et al. (2012) exposed athletes to either live-high-train-low for 16 h daily at a simulated 3000 m or else a blinded, placebo nonhypoxic exposure and training for 4 weeks. Notably, high inter-individual variability in tHb-mass was reported in the live-high-train-low group, with five of ten exhibiting increases but three significantly decreasing tHb-mass. In a review of the effects of endurance training at altitude, Saunders et al. (2009a) also concluded a general trend of progressively increasing tHb-mass with exposure time to hypoxia; however, in all modes of training (e.g., live-high-train-high and live-high-train-low), a wide inter-individual variation in responses existed. Our data suggest a similar wide variability in response also prevails at high and extreme altitudes in climbers, at least during self-supported expeditions. The genetic bases for a phenotypic dichotomy remain open to debate, as an examination of eight different genes involved in erythropoietin regulation did not produce a significant association with erythropoietin response to 24 h of hypoxia (Jedlickova et al., 2003).

Limitations

As with any field study, research design and expedition imperatives must be balanced. Unlike laboratory studies, sample size was constrained to the nine expedition members and could not be simply increased to strengthen statistical power. This may have especially influenced our ability to truly discern the effects of such prolonged expeditions on tHb-mass. The participants were all experienced climbers performing a particularly strenuous self-supported climb, so the results may have been impacted by the heavy exercise and thus not directly generalizable to the normal population of altitude trekkers or guided climbers. While all participants were members of the same expedition, it is recognized that the actual level of exercise, nutrition, and hypoxia exposure was not controlled across the team members. The study design does not completely eliminate the possibility that the prolonged hypoxic tolerance post-expedition were due to improvements in overall fitness or in specific adaptation to the physical demands of mountaineering. A maximal aerobic fitness test was not performed on the climbers post-expedition, as scheduling was prioritized towards a timely testing of the exercise-hypoxic protocol. The exercise-hypoxia exposure had graded hypoxia being the primary stressor, with exercise workload remaining constant. Tolerance time greatly increased in 8 out of 9 participants, which we assume to be due to physiological mechanisms, and not learning effects, as all participants were familiar with exercise testing in laboratory settings from their previous expeditions. Finally, it is possible that de-acclimatization was already occurring during the inevitable delay between the climb's completion and getting the participants back to Finland and post-expedition testing. While Heinicke et al. (2005) reported that tHb-mass returned to baseline values 16 days in elite biathletes following an altitude training camp (2050 m), Prommer et al. (2010) found that, while tHb-mass in elite Kenyan runners eventually decreased upon moving to sea level, this decrease was not evident over the initial 14 days of descent.

Conclusions

This study investigated the physiological adaptations to a self-supported Everest expedition, featuring both chronic

hypobaric hypoxia and heavy exercise. The primary finding was that tolerance to progressive hypoxia during steady-state exercise improved post-expedition. In exploring the mechanisms for this improved tolerance, we conclude that, following chronic hypobaric hypoxic acclimatization: 1) ventilatory chemosensitivity to changes in arterial saturation was significantly elevated; 2) arterial oxygenation levels were higher, but only at simulated altitudes 4800 m and beyond; 3) a lower level of cerebral deoxygenation was observed post-expedition, suggesting less of a mismatching in the ratio between cerebral oxygen delivery and utilization, while active muscle oxygenation did not change to a major degree; and 4) total hemoglobin mass showed tendencies for an overall increase but also great inter-individual variability. The magnitude of acute hypoxic ventilatory response did not influence the response of cerebral or active muscle oxygenation to acute exercise-hypoxia.

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Author Disclosure Statement

The authors declare that they have no competing financial interests.

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