HIGHLIGHTED TOPIC | Hypoxia

AltitudeOmics: enhanced cerebrovascular reactivity and ventilatory response to CO2 with high-altitude acclimatization and reexposure

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1Institute of Sports Sciences, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland; 2Lemanic Neuroscience Doctoral School, University of Lausanne, Lausanne, Switzerland; 3Altitude Research Center, Department of Emergency Medicine, University of Colorado Denver, Aurora, Colorado; 4Department of Biology; University of Colorado, Colorado Springs, Colorado; and 5Department of Human Physiology, University of Oregon, Eugene, Oregon

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Fan JL, Subudhi AW, Evero O, Bourdillon N, Kayser B, Lovering AT, Roach RC. AltitudeOmics: enhanced cerebrovascular reactivity and ventilatory response to CO2 with high-altitude acclimatization and reexposure. J Appl Physiol 116: 911–918, 2014. First published December 19, 2013; doi:10.1152/japplphysiol.00704.2013.—The present study is the first to examine the effect of high-altitude acclimatization and reexposure on the responses of cerebral blood flow and ventilation to CO2. We also compared the steady-state estimates of these parameters during acclimatization with the modified rebreathing method. We assessed changes in steady-state responses of middle cerebral artery velocity (MCAv), cerebrovascular conductance index (CVCi), and ventilation (V̇e) to varied levels of CO2 in 21 lowlanders (9 women; 21 ± 1 years of age) at sea level (SL), during initial exposure to 5,260 m (ALT1), after 16 days of acclimatization (ALT16), and upon reexposure to altitude following either 7 (POST7) or 21 days (POST21) at low altitude (1,525 m). In the nonacclimatized state (ALT1), MCAv and V̇e responses to CO2 were elevated compared with those at SL (by 79 ± 75% and 14.8 ± 12.3 l/min, respectively; P = 0.004 and P = 0.011). Acclimatization at ALT16 further elevated both MCAv and V̇e responses to CO2 compared with ALT1 (by 89 ± 70% and 48.3 ± 32.0 l/min, respectively; P < 0.001). The acclimatization gained for V̇e responses to CO2 at ALT16 was retained by 38% upon reexposure to altitude at POST7 (P = 0.004 vs. ALT1), whereas no retention was observed for the MCAv responses (P > 0.05). We found good agreement between steady-state and modified rebreathing estimates of MCAv and V̇e responses to CO2 across all three time points (P < 0.001, pooled data). Regardless of the method of assessment, altitude acclimatization elevates both the cerebrovascular and ventilatory responsiveness to CO2. Our data further demonstrate that this enhanced ventilatory CO2 response is partly retained after 7 days at low altitude.

cerebral blood flow; cerebral CO2 reactivity; rebreathing; altitude acclimatization

THE ABILITY TO MAINTAIN ADEQUATE oxygen transport to the brain by cerebral blood flow (CBF) in hypoxic environments is vital. The CBF responsiveness to CO2, termed cerebrovascular CO2 reactivity, provides a useful, noninvasive index of cerebrovascular function (3, 19). To date, only a handful of studies have investigated the effect of acclimatization to high altitude on cerebrovascular CO2 reactivity (1, 16, 17, 24, 30, 49). It is difficult to interpret the findings from these studies due to the timing of measurements at high altitude (1, 16, 17, 24, 25), the confounding effects of previous high altitude exposure (1), artificial normobaric hypoxia (28, 46), and the method used to assess reactivity (24, 30, 49). Data obtained by Fan et al. (16, 17) on subjects at different stages of altitude acclimatization suggest that cerebrovascular CO2 reactivity is elevated with prolonged exposure to high altitude when using a modified rebreathing technique. In contrast, Lucas et al. (30) reported, using a steady-state technique (poikilocapnic hypoxia), reduced cerebrovascular CO2 reactivity in the same subjects assessed at the end of a 14-day stay at 5,050 m. More recently, Rupp et al. (49) reported a reduced cerebrovascular CO2 reactivity during steady-state hypoxic hypercapnia following 5 days at 4,350 m. Thus the effect of altitude acclimatization on cerebrovascular CO2 reactivity remains unclear.

In addition, it is unknown whether and for how long changes in cerebrovascular CO2 reactivity from acclimatization persist after descent. Repetitive 7-mo exposures to high altitude were reported to improve arterial O2 saturation (SaO2), lower resting heart rate (HR), and decrease susceptibility to acute mountain sickness (AMS) upon subsequent reexposures (59). Remarkably, these prior exposure adaptations persisted despite a 5-mo deacclimatization period. The specific effect of high-altitude reexposure on cerebrovascular and ventilatory responsiveness to CO2 has yet to be examined.

Changes in cerebrovascular CO2 reactivity with high-altitude acclimatization depend on the method of assessment. At sea level, the steady-state method results in higher cerebrovascular CO2 reactivity (40–42) and lower ventilatory CO2 sensitivity (6, 18, 23, 55) compared with the modified rebreathing test. These differences have been attributed to the presence of a PCO2 gradient (between alveolar, arterial, and cerebrospinal fluid compartments) during the steady-state method, which is supposedly abolished or minimized during rebreathing (6). Meanwhile, elevated basal V̇e and subsequent underestimation of the ventilatory CO2 sensitivity has been proposed as one possible explanation for lower steady-state estimates (34). No studies have directly compared the steady-state and modified rebreathing test estimates of cerebrovascular and ventilatory CO2 responsiveness following ascent or acclimatization to high altitude.

The purpose of the present study was therefore twofold: first, we wished to assess the effect of altitude exposure on cerebro-
vascular and ventilatory responsiveness to CO\textsubscript{2} in acute conditions after acclimatization and upon reexposure to high altitude after a period spent at low altitude; second, we wished to compare the steady-state and modified rebreathing methods for assessing the ventilatory and cerebrovascular responsiveness to CO\textsubscript{2} at high altitude.

**METHODS**

**Subject Recruitment and Screening**

This study was conducted as part of the AltitudeOmics project. Following institutional ethics approval, young (19–23 years old), healthy, sea-level residents were recruited from the greater Eugene, Oregon, area (elevation 130 m). Potential subjects were screened to exclude anyone who was born or had lived at altitudes >1,500 m for more than 1 year or had traveled to altitudes >1,000 m in the past 3 mo. A detailed description of subject recruitment procedures, including inclusion and exclusion criteria, has been presented elsewhere (54).

**Ethical Approval**

The study was performed according to the Declaration of Helsinki and was approved by the institutional review boards of the University of Colorado and the University of Oregon, and by the Human Research Protection Office of the U.S. Department of Defense. All participants were informed regarding the procedures of this study, and written informed consents were obtained prior to participation.

**Experimental Design**

After familiarization with the experimental procedures outlined below (visit 1), the subjects underwent experimental trials near sea level (SL) (130 m; barometric pressure 749 mmHg) and three times at high altitude (5,260 m, Mt. Chacaltaya, Bolivia; barometric pressure 639 mmHg). An overview of the entire experimental design and protocol has been described in detail elsewhere (54).

**Experimental Protocol**

For each subject, all ALT measurements were carried out around the same time of day to minimize any confounding effect of circadian rhythm. Measurements were taken upon arrival at ALT1 to minimize the influence of AMS. Likewise, no symptoms of AMS were observed at ALT16 or POST7.

For this study, following 10–15 min of quiet rest in a seated position, each experimental testing session consisted of 1) instrumentation, 2) 10 min in room air at baseline, and 3) cerebrovascular CO\textsubscript{2} reactivity tests. The cerebrovascular CO\textsubscript{2} reactivity tests consisted of 1) 10 min with end-tidal P\textsubscript{CO\textsubscript{2}} (P\textsubscript{ET\textsubscript{CO\textsubscript{2}}} \textsubscript{t}) clamped at 40 mmHg; 2) 3 min of voluntary hyperventilation to lower P\textsubscript{ET\textsubscript{CO\textsubscript{2}}} \textsubscript{t} to ~20 mmHg; 3) the modified rebreathing test (details below); and 4) 3 min with P\textsubscript{ET\textsubscript{CO\textsubscript{2}}} \textsubscript{t} clamped at 50 mmHg. The entire cerebrovascular CO\textsubscript{2} reactivity protocol was carried out in a background of hyperoxia (end-tidal P\textsubscript{O\textsubscript{2}} [P\textsubscript{ETO\textsubscript{2}}] >250 mmHg).

**Modified Rebreathing Method**

The modified rebreathing method is well established for assessing both ventilatory and cerebrovascular CO\textsubscript{2} reactivities (14, 16, 34, 41). By using hyperoxia (P\textsubscript{ETO\textsubscript{2}} >250 mmHg) the test minimizes the output of peripheral chemoreceptors (11, 21), and the ventilatory response to the modified rebreathing method can thus be interpreted as the ventilatory CO\textsubscript{2} sensitivity primarily from the central chemoreflex. The details of the modified rebreathing method have been previously described in Fan et al. (16, 17). The rebreathing bag was filled with gas to achieve inspired P\textsubscript{CO\textsubscript{2}} and P\textsubscript{O\textsubscript{2}} of 0 mmHg and 300 mmHg, respectively, at each altitude. Subjects were instructed to hyperventilate for 3 min (part 2) to lower and then maintain P\textsubscript{ET\textsubscript{CO\textsubscript{2}}} at 20 mmHg at both sea level and 5,260 m (in background P\textsubscript{ETO\textsubscript{2}} >250 mmHg). Subjects were then switched to the rebreathing bag, and following two initial deep breaths to mix the gas from the bag with that in the respiratory system, they were instructed to breathe ad libitum (part 3). The rebreathing tests were terminated when P\textsubscript{ET\textsubscript{CO\textsubscript{2}}} reached 50 mmHg, P\textsubscript{ETO\textsubscript{2}} dropped below 200 mmHg, or the subject reached the end of his or her hypcapnic tolerance.

**Measurements**

**Cerebrovascular variables.** Middle cerebral artery velocity (MCA\textsubscript{v}, an index of cerebral blood flow) was measured in the left middle cerebral artery using a 2-MHz pulsed Doppler ultrasound system (ST3; Spencer Technology, Seattle, WA). The Doppler ultrasound probe was positioned over the left temporal window and held in place with an adjustable plastic headband (Med 600 Headframe; Spencer Technology). The signal was acquired at depths ranging from 43 to 54 mm. Signal quality was optimized, and an M-mode ultrasound image was recorded to facilitate subsequent probe placements. Peripheral saturation was measured on the right side of the forehead by pulse oximetry (N-200; Nellcor, Hayward, CA).

**Cardiovascular variables.** Beat-to-beat mean arterial blood pressure (MAP) was measured from an arterial catheter inserted in a radial artery, and connected to a calibrated, fluid-filled, disposable pressure transducer positioned at the level of the heart (DELTRAN II; Utah Medical, Salt Lake City, UT). HR was determined using three-lead electrocardiography systems (ADInstruments BioAmp & Micromaxx; SonoSite, Bothell, WA). Cerebrovascular conductance index (CVC\textsubscript{i}) was calculated using the equation CVC\textsubscript{i} = MCA\textsubscript{v}/MAP and normalized to values obtained at a P\textsubscript{ET\textsubscript{CO\textsubscript{2}}} of 20 mmHg, and expressed as percentage change.

**Respiratory variables.** VE was measured using a pneumotachograph (Universal Ventilation Meter; Vacu-Med, Ventura, CA; Ultima series; Medgraphics CPX, Minneapolis, MN) and expressed in units adjusted to body temperature and pressure, saturated (BTPS). P\textsubscript{ETO\textsubscript{2}} and P\textsubscript{ET\textsubscript{CO\textsubscript{2}}} were measured using fast-responding gas analyzers (O\textsubscript{2}Cap Oxygen analyzer; Oxigraf, Mountain View, CA). The pneumotachograph was calibrated using a 3-liter syringe (Hans-Rudolph 5530) and the gas analyzers were calibrated using gas mixtures of known concentrations of O\textsubscript{2} and CO\textsubscript{2} prior to each testing session.

**Arterial blood gas variables.** An arterial catheter (20–22 gauge) was placed into a radial artery and blood samples (2 ml) were taken over approximately five cardiac cycle periods. Core body temperature was telemetrically recorded from an ingested pill (CorTemp; HQInc, Palmetto, FL). All samples were analyzed immediately for arterial pH, P\textsubscript{O\textsubscript{2}} (P\textsubscript{A\textsubscript{O\textsubscript{2}}}), P\textsubscript{CO\textsubscript{2}} (P\textsubscript{A\textsubscript{CO\textsubscript{2}}}) (Rapidlab 248; Siemens Healthcare Diagnostics, Munich, Germany), hemoglobin concentration, and O\textsubscript{2} saturation (S\textsubscript{a\textsubscript{O\textsubscript{2}}}) (Radiometer OSM3; Radiometer Medical ApS, Copenhagen, Denmark). The blood gas values were analyzed in triplicate and temperature-corrected (26, 53). Arterial bicarbonate concentration
([HCO₃⁻]) was subsequently calculated using the Henderson-Hasselbalch equation.

Data Acquisition

All analog data were sampled and recorded at 200 Hz on a personal computer for off-line analysis (Powerlab 16/30; ADInstruments, Bella Vista, Australia).

Data Analysis

Steady-state responses. Because the subjects could not tolerate PetCO₂ clamping at 50 mmHg at ALT16, the steady-state MCAv-CO₂, MAP-CO₂, and CVCi-CO₂ slopes were estimated from the difference in mean MCAv, MAP, and CVCi at the end of 20 and 40 mmHg PetCO₂, clamping (20-s averages) and plotted against the change in PetCO₂ between these two conditions across all time points (SL, ALT1, ALT16, POST7, and POST21). The absolute value of Ve at clamp 40 mmHg was used as an estimate of steady-state Ve responsiveness to CO₂, because voluntary hyperventilation was necessary to reduce PetCO₂ to 20 mmHg.

Modified rebreathing. The rebreathing data were first reduced to 1-s averages across the entire rebreathing period. The Ve-CO₂ slopes were analyzed using a specially designed program (Analyze Ve Rebreathing programme rev11; University of Toronto, Toronto, ON, Canada) as previously described (15, 16, 34). The MCAv-CO₂ slopes were analyzed using a commercially available graphing program (Prism 5.0d; GraphPad Software, San Diego, CA), whereby segmental linear regression (least squares fit) was used to estimate the MCAv-CO₂ slopes, CVCi-CO₂ slope, and Ve at 40 mmHg were analyzed using a specially designed program (Analyse Ve algorithm) we used the equation MCAv = a + (b/[1 + exp(−(PetCO₂ − c)/d)]), where MCAv is the dependent variable in cm/s, PetCO₂ is the independent variable in mmHg, a is the minimum MCAv determined from the mean MCAv of the hypocapnic (hyperventilation) region, b is the maximum MCAv value, c is the midpoint value of MCAv, and d is the range of the linear portion of the sigmoidal curve (inverse reflection of the slope of the linear portion).

We found good agreement in the MCAv-CO₂ slope obtained from these two models (R² = 0.71). However, due to the range of PetCO₂ used in this study, segmental linear regression generally provided better fit across all conditions, whereas the sigmoidal curve model was the preferred model for only 12 out of 58 trials. As such, only the MCAv-CO₂ slopes obtained using the segmental linear model are presented.

Statistical Analysis

Due to logistical impacts on planning and transportation, not all subjects were able to participate in all high-altitude studies. See the Figs. 1–3 and Table 1 for complete sample size reporting for each procedure. Most data are reported as the improvement over the time of acclimatization (change from ALT1 to ALT16) and as the amount of that improvement that was retained after time at low altitude, calculated as % retention = (POST7 or POST21 − ALT1)/(ALT16 − ALT1) · 100 (5). The effects of altitude acclimatization and reexposure (between SL, ALT1, ALT16, POST7, and POST21) on the steady-state MCAv-CO₂ slope, CVCi-CO₂ slope, and Ve at 40 mmHg were analyzed using a mixed-model linear regression (IBM SPSS Statistics version 21; IBM, Armonk, NY). To assess the effects of altitude acclimatization (between SL, ALT1, and ALT16) on the rebreathing estimates of MCAv-CO₂ and Ve-CO₂ slopes, we used mixed-model linear regression analysis (diagonal repeated covariance assumed). The interactions between variables of interest were assessed using correlational (Pearson) analysis (IBM SPSS Statistics version 21). Data are shown as mean ± SD. Results were considered significant at α < 0.05. Trends were consider at the α < 0.10 level. A priori power calculations (α = 0.05, β = 0.20) were used to determine sample size and limit type II error.

Fig. 1. Changes in steady-state estimates of cerebrovascular, cardiovascular, and ventilatory responsiveness to CO₂ with acclimatization and reexposure to 5,260 m. Values are mean ± SD. *Different from SL (P < 0.05); †different from ALT1 (P < 0.05); §different from ALT16 (P < 0.05).
RESULTS

Detailed baseline characteristics of the 21 (9 women; age 21 ± 1 years) subjects participating in AltitudeOmics are presented elsewhere (54). All 21 subjects completed the protocol at SL. Due to logistical issues, 4 of 21 subjects were unable to complete the entire experimental protocol at ALT1. Upon reexposure to altitude, 14 of 14 subjects completed the protocol at POST7, and 5 of 7 completed the protocol at POST21. No comparison was carried out between ALT1 and POST21 due to the low number of subjects.

Resting Variables

The resting variables across acclimatization and reexposure have already been reported in detail elsewhere (54) and will not be reproduced in this paper.

Steady-State Method

Acclimatization. Compared with SL, the steady-state MCAv-CO₂ slope was elevated at ALT1 (by 79 ± 70%; P < 0.001), and remained higher at ALT16 (by 93 ± 81%; P < 0.001 vs. SL, no difference with ALT1). Ve at 40 mmHg was elevated at ALT1 compared with SL (by 14.8 ± 12.3 l/min; P = 0.011), and further elevated at ALT16 (by 48.3 ± 32.0 l/min vs. ALT1; P < 0.001).

Reexposure. Upon reexposure to altitude, it appears that the acclimatization gained in the steady-state MCAv-CO₂ slope was not retained at POST7 (P = 0.145 vs. ALT1). Compared with ALT16, the steady-state MCAv-CO₂ slope was lowered at both POST7 and POST21 (P = 0.029 and P = 0.003, respectively), but nevertheless remained higher compared with SL (P < 0.001 and P = 0.024, respectively). Similarly, 49% of the acclimatization gained in the MAP-CO₂ slope was retained at POST7. Specifically, the MAP-CO₂ slope remained higher at ALT16 compared with ALT1 (P = 0.005). Compared with ALT16, the MAP-CO₂ slope was lowered at both POST7 and POST21 (P < 0.001 for both). Nevertheless, the MAP-CO₂ slope was higher at POST7 and POST21 compared with SL (P < 0.001 and P = 0.020, respectively). In contrast, no difference was observed in the CVCi-CO₂ slope at POST7 compared with ALT1 or ALT16 (P = 0.980 and P = 0.804, respectively), but it remained higher compared with SL (P < 0.001). Likewise, the CVCi-CO₂ slope tended to remain higher.

Fig. 2. Relationship between standard basic excess and steady-state cerebrovascular, ventilatory, and cardiovascular responsiveness to CO₂ with acclimatization to altitude. *Significant correlations (P < 0.05).

Fig. 3. Comparison of steady-state and rebreathing estimates of cerebrovascular and ventilatory responsiveness of CO₂ with acclimatization to 5,260 m. *Significant correlations (P < 0.05).
at POST21 compared with SL (P = 0.058) but was not different from ALT16 (P = 0.715).

Upon reexposure, the effect of acclimatization on $V_e$ at 40 mmHg was retained by 38% at POST7 (P = 0.004 vs. ALT1), Compared with ALT16, $V_e$ at 40 mmHg was lower at POST7 and POST21 (P = 0.001 and P < 0.001, respectively), but these values remained higher compared with SL (P < 0.001 and P = 0.001, respectively).

**Modified Rebreathing Method**

Similar to the steady-state method, the rebreathing MCAv-CO$_2$ slope was elevated at ALT1 (by 137% ± 117%; P < 0.001), and further elevated at ALT16 (by 35% ± 33% vs. ALT1; P = 0.040) (Table 1). The rebreathing $V_e$-CO$_2$ slope was elevated at ALT1 compared with SL (by 1.61 ± 1.14 l·min$^{-1}$·mmHg$^{-1}$; P = 0.038), and further elevated at ALT16 (by 2.86 ± 2.61 l·min$^{-1}$·mmHg$^{-1}$ vs. ALT1; P = 0.004). The ventilatory recruitment threshold was lowered at ALT1 (by 4.4 ± 4.0 mmHg; P < 0.001 vs. SL) and further lowered at ALT16 (by 4.4 ± 3.2 mmHg vs. ALT1; P < 0.001).

**Acid-Base Buffering Capacity Correlations**

Based on previous findings (16), we performed correlations between the pooled steady-state data with [HCO$_3^-$] and found that resting [HCO$_3^-$] correlated with the steady-state MCAv-CO$_2$ slope (R = -0.771) and $V_e$ at 40 mmHg (R = -0.723; P < 0.001 for both) (Fig. 2).

**Steady-State vs. Modified Rebreathing**

We observed correlations between the steady-state and rebreathing MCAv-CO$_2$ slope at SL (R = 0.609; P = 0.003), ALT1 (R = 0.817; P < 0.001), and ALT16 (R = 0.596; P = 0.007), whereas the pooled MCAv-CO$_2$ slopes (combined SL, ALT1, and ALT16) between the two methods also correlated well (R = 0.860; P < 0.001) (Fig. 3). Likewise, there were significant correlations between $V_e$ at 40 mmHg and the rebreathing $V_e$-CO$_2$ slope at SL (R = 0.476; P = 0.029), ALT1 (R = 0.506; P = 0.038), and ALT16 (R = 0.927; P < 0.001), whereas the pooled ventilatory data across all time points also correlated (R = 0.904; P < 0.001).

**DISCUSSION**

The present study is the first to assess the effect of altitude acclimatization and reexposure on cerebrovascular CO$_2$ reactivity using both the steady-state and modified rebreathing methods. We demonstrate that cerebrovascular CO$_2$ reactivity was elevated immediately upon arrival at 5,260 m and is further elevated following 16 days of acclimatization regardless of the method of assessment. In addition, we found that cerebrovascular and ventilatory responsiveness to CO$_2$ remains elevated upon reexposure to altitude, despite 7 or 21 days at low altitude. Because these changes in cerebrovascular and ventilatory responsiveness to CO$_2$ correlated with the changes in resting arterial [HCO$_3^-$] across all time points, we speculate that these changes might be partly due to an altered pH buffering capacity associated with exposure to high altitude. Our data thus demonstrate that the changes in cerebrovascular and ventilatory control gained due to altitude acclimatization over a period of 16 days are partially preserved upon subsequent exposure to altitude, at least for up to a period of 3 wk spent at low altitude.

**Effects of Acclimatization on Cerebrovascular CO$_2$ Reactivity**

Our findings extend those from Fan et al. (16, 17) by demonstrating that the MCAv-CO$_2$ slope is elevated upon arrival at 5,260 m and further elevated following 16 days of acclimatization (Fig. 1A). Importantly, previous studies by Fan et al. (16, 17) assessed MCAv-CO$_2$ slope in subjects who spent 8 days ascending to 5,050 m, whereas the subjects in the present study ascended rapidly to altitude (~3 h), thus making direct comparison difficult. Our findings contradict those of Lucas et al. (30), who found that the MCAv-CO$_2$ slope was initially elevated at 5,050 m, but had returned toward sea level values following 2 wk at 5,050 m. However, because $P_{ETO_2}$ was not controlled, the MCAv-CO$_2$ slopes reported by Lucas et al. (30) reflect MCAv changes from polikilocapnic hypoxia (room air breathing at 5,050 m: $P_{ETO_2}$ ~ 48 mmHg and $P_{ETCO_2}$ 26–22 mmHg) to hypercapnic hypoxia ($P_{ETO_2}$ > 310 mmHg and $P_{ETCO_2}$ ~ 30 mmHg), and thus do not represent isolated reactivity to CO$_2$. Rupp et al. (49) recently found the MCAv response to steady-state hypoxic hypercapnia ($P_{ETO_2}$ = 55 mmHg) to be reduced following 5 days at 4,350 m. Therefore, discrepancies between findings by Rupp et al. (49) and those of the present study can be attributed the differences in $P_{ETO_2}$ (55 mmHg vs. >200 mmHg), altitude (4,350 m vs. 5,260 m), and acclimatization state of the subjects (5 days vs. 16 days). The results from the present study demonstrate for the first time that cerebrovascular CO$_2$ reactivity per se is enhanced with acclimatization to high altitude when studied using a background level of hyperoxia. Furthermore, discrepancies between studies

Table 1. Cerebrovascular and ventilatory reactivity parameters during the steady-state and modified rebreathing

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SL (n = 21)</th>
<th>ALT1 (n = 17)</th>
<th>ALT16 (n = 20)</th>
<th>POST7 (n = 14)</th>
<th>POST21 (n = 5)</th>
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<tbody>
<tr>
<td>Steady-state</td>
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<tr>
<td>MCAv-PaCO$_2$ slope (cm$^{-1}$·mmHg$^{-1}$)</td>
<td>1.19 ± 0.42</td>
<td>2.16 ± 1.05*</td>
<td>3.39 ± 0.89*‡</td>
<td>2.68 ± 0.88*‡</td>
<td>2.06 ± 0.57*‡</td>
</tr>
<tr>
<td>CVCG-PaCO$_2$ slope (%/mmHg)</td>
<td>3.35 ± 1.21</td>
<td>5.87 ± 2.60*</td>
<td>5.75 ± 1.85*</td>
<td>5.89 ± 1.23*</td>
<td>5.41 ± 1.78*</td>
</tr>
<tr>
<td>MAP-PaCO$_2$ slope (l/min)</td>
<td>0.03 ± 0.24</td>
<td>0.28 ± 0.19*</td>
<td>1.06 ± 0.45*</td>
<td>0.56 ± 0.29*‡</td>
<td>0.32 ± 0.18*‡</td>
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<tr>
<td>V$_e$ at 40 mmHg (l/min)</td>
<td>19.15 ± 4.89</td>
<td>34.06 ± 12.23*</td>
<td>80.05 ± 32.32‡</td>
<td>49.03 ± 13.68‡†</td>
<td>43.25 ± 7.56‡†</td>
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<td>Modified rebreathing</td>
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<tr>
<td>MCAv-PETCO$_2$ slope (cm$^{-1}$·mmHg$^{-1}$)</td>
<td>1.34 ± 0.60</td>
<td>2.95 ± 1.11*</td>
<td>3.67 ± 0.87*</td>
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<tr>
<td>$V_e$-CO$_2$ slope (l·min$^{-1}$·mmHg$^{-1}$)</td>
<td>1.90 ± 0.81</td>
<td>3.49 ± 1.51*</td>
<td>6.28 ± 3.56*</td>
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<tr>
<td>$V_e$ recruitment threshold (mmHg)</td>
<td>38.7 ± 5.4</td>
<td>33.7 ± 3.7*</td>
<td>29.2 ± 2.1*‡</td>
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</table>

All values are mean ± SD. SL, sea level; ALT1, day 1 at high altitude; ALT16, day 16 at high altitude; POST7, reexposure following 7 days at low altitude; POST21, reexposure following 21 days at low altitude. *Different from SL (P < 0.05); †different from ALT1 (P < 0.05); ‡different from ALT16 (P < 0.05).
highlight how methodological differences can yield vastly different results. Thus future studies are warranted to clarify the effect of hypoxic and hyperoxic background on assessing cerebrovascular functions at both sea level and following ascent to high altitude.

**Altered Acid-Base Buffering Capacity?**

During altitude acclimatization, there is a progressive and parallel reduction in arterial and cerebrospinal fluid (CSF) bicarbonate concentration, which serves to compensate for the changes in pH associated with hyperventilation-induced hypocapnia (12, 13, 20). These changes in acid-base buffering capacity, in both the arterial and CSF compartments, would lead to a greater rise in arterial and CSF [H+] for a given rise in PaCO₂. In support of this notion, lowering CSF bicarbonate concentration elevates the cerebrovascular CO₂ reactivity in an anesthetized dog model (27), whereas bicarbonate infusion increases cerebral perfusion pressure in patients with posttraumatic head injury (9), elevates cerebral blood volume in preterm infants (57), and lowers ventilation in healthy exercising humans at SL (44). As such, it has been suggested that the MCAv responses to CO₂ at high altitude are linked to changes in arterial acid-base balance (16, 25). In the present study, we observed concomitant increases in cerebrovascular and ventilatory responsiveness to CO₂ with acclimatization to high altitude and reexposure (Fig. 1), which occurred in parallel to the changes in [HCO₃⁻] (Fig. 2). While such correlations do not imply causality, the possible role for acid-base status changes on cerebrovascular and ventilatory responsiveness to CO₂ at high altitude remains to be further studied.

**Interaction Between Cerebrovascular and Ventilatory Responsiveness to CO₂**

Interaction between cerebrovascular CO₂ reactivity and central chemoreceptor activation was first alluded to by Heyman et al. (22) and has been subsequently expanded upon by others (10, 16–18, 38, 43, 60–62). It was postulated that changes in cerebrovascular CO₂ reactivity affect the stability of the ventilatory response to CO₂ by modulating the degree of H⁺ washout at the level of the central chemoreceptor (38). Accordingly, a blunted cerebrovascular CO₂ reactivity would lead to less central H⁺ washout and subsequently greater central chemoreceptor activation. Conversely, an enhanced cerebrovascular CO₂ reactivity would result in lower central H⁺ and therefore lower ventilatory CO₂ sensitivity. In agreement with previous altitude studies (16, 17), we observed concomitant increases cerebrovascular and ventilatory responsiveness to CO₂ (Fig. 1). These findings seem to contradict the modulating role of cerebrovascular CO₂ reactivity on central chemoreceptor activation, possibly due to other overriding factors such as enhanced central chemosensitivity and changes in acid-base balance associated with ascent to high altitude. Future work is necessary to further unravel the interaction between the regulation of cerebral blood flow and ventilation.

**Going Back Up**

Despite the large body of literature regarding high-altitude acclimatization over the past century, the effect of prior exposure on physiological parameters during subsequent exposures is not well documented. Most attention has focused on the effect of a recent altitude exposure on the risk for AMS (7, 31, 45, 51) or the rate of ascent (56). However, the dose of previous altitude exposure and acclimatization were generally not controlled in these studies. Wu et al. (59) found a progressive reduction in the incidence of AMS, lower HR, and higher SpO₂ in lowland railroad workers over the course of several 7-mo exposures to high altitude interspersed with 5 mo spent at low altitude. Similarly, MacNutt et al. (32) found faster rate of ascent, lower AMS, and higher SpO₂ in trekkers with a recent altitude exposure compared with altitude-naïve trekkers, despite a 7- to 30-day deacclimatization period. In the present study, we compared the cerebrovascular and ventilatory responsiveness to CO₂ with acclimatization and upon reexposure to 5,260 m following a period of either 7 or 21 days at low altitude. We found that 38% of the gain in ventilatory response to CO₂ over acclimatization was retained at POST7 (Fig. 1C), whereas essentially none of the gain in MCAv-CO₂ reactivity over acclimatization was retained at POST7 (Fig. 1A). Regardless of the underpinning mechanism(s), our findings suggest that the effect of previous altitude acclimatization over 16 days on the ventilatory response to CO₂ is partially retained after 7 days at low altitude, whereas it is reversed in the cerebrovascular response to CO₂. Our data extend findings by Muza et al. (36) showing that ventilatory acclimatization gained at 4,300 m is retained following 8 days spent at low altitude. Because we found the CVCi-CO₂ slope to be consistently elevated by 60–80% across all time points (Fig. 1D), whereas the changes in MAP-CO₂ slope closely follow the changes in MCAv-CO₂ slope (Fig. 1B), we speculate that the changes in MCAv-CO₂ slope at high altitude can be primarily accounted for by an enhanced sensitivity of the cerebral vessels to CO₂, whereas the remainder can be attributed to an enhanced perfusion pressure response.

**Steady-State or Modified Rebreathing Method?**

There has been much debate over the use of the steady-state or the modified rebreathing method for the assessment of cerebrovascular and ventilatory control, and attempts at consensus have produced no uniform agreement [(18, 40), also see (2, 14) for reviews]. The steady-state ventilatory responses to CO₂ were found to be either similar (34, 37, 40–42, 47) or lower (6, 18, 23, 55) compared with rebreathing estimates, whereas steady-state cerebrovascular CO₂ reactivity has been shown to be consistently higher than rebreathing values (18, 40–42). The present study demonstrates that the changes in cerebrovascular and ventilatory CO₂ responsiveness with altitude acclimatization were similar between the steady-state and the modified rebreathing method (Table 1), possibly due to tight control of arterial PCO₂ and PO₂ with our end-tidal clamping setup. Moreover, we observed strong correlations in these parameters between the two methods across all time points (Fig. 3). We therefore conclude that both methods can be used to assess the changes in cerebrovascular and ventilatory responses to CO₂ with high altitude exposure and acclimatization, provided that the level of CO₂ is comparable across all the conditions, under identical levels of background O₂.
Limitations

Although the present study provided the opportunity to assess the effects of acclimatization and reexposure to 5,260 m on cerebrovascular CO$_2$ reactivity, an important methodological consideration should be acknowledged when interpreting our findings. In the present study, transcranial Doppler ultrasound (TCD) was used to measure MCAv as an index of global CBF changes during initial exposure, acclimatization, and subsequent reexposure to 5,260 m. This is based on the assumption that 1) the MCA carries approximately upward of 80% of the overall blood flow to the respective hemisphere (29); 2) changes in MCAv reflect changes in global CBF (8, 52); 3) the changes in MCAv in response to PaCO$_2$ changes are comparable to the changes in global CBF (8, 52); and 4) the diameter of the MCA does not change during the observed changes in arterial blood gases (52). In support, MCAv has been shown to reflect changes in CBF assessed with the direct Fick method, at least during initial exposure to high altitude (33, 35, 48).

Recent findings by Wilson et al. (58) indicate that the diameter of the MCA, as measured using TCD, varies depending on the altitude (e.g., 5.30 mm at 75 m, 5.51 mm at 3,500 m, 5.23 mm at 5,300 m, and 9.34 mm at 7,950 m). Importantly, the results reported by Wilson et al. (58) demonstrate that the MCA diameter remains relatively unchanged up to 5,300 m. It should be noted that the MCA diameters measured with TCD in that study were 80–90% greater than the values obtained using magnetic resonance imaging in the same subjects. Because our measurements were carried out in background hypoxia (P$_{ET}$CO$_2$ >300 mmHg), it seems unlikely that our cerebral blood velocity values would be confounded by any effect of hypoxia-induced vasodilatation of the MCA. Further studies are needed to evaluate MCAv responses to CO$_2$ while holding P$_{ET}$CO$_2$ at consistent levels of hypoxia.

Conclusion

Findings from the present study clearly show that both cerebrovascular and ventilatory responsiveness to CO$_2$ is elevated upon arrival at high altitude and further elevated with acclimatization. We demonstrate for the first time that this effect of high-altitude acclimatization on the ventilatory response to CO$_2$ is partially retained after a period at low altitude, whereas prior acclimatization has no effect on the cerebrovascular response to CO$_2$. Our data suggest that the increased cerebrovascular CO$_2$ reactivity with acclimatization may be accounted for by the changes in acid-base balance in the blood and possibly the CSF compartment.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


