



AltitudeOmics: Resetting of Cerebrovascular CO₂ Reactivity Following Acclimatization to High Altitude

Jui-Lin Fan^{1,2*}, Andrew W. Subudhi^{3,4}, James Duffin^{5,6,7}, Andrew T. Lovering⁸, Robert C. Roach^{3,4} and Bengt Kayser^{9,10}

¹ Centre for Translational Physiology, University of Otago, Wellington, New Zealand, ² Department of Surgery and Anaesthesia, University of Otago, Wellington, New Zealand, ³ Department of Emergency Medicine, Altitude Research Center, University of Colorado Denver, Aurora, CO, USA, ⁴ Department of Biology, University of Colorado Colorado Springs, Colorado Springs, CO, USA, ⁵ Department of Physiology, University of Toronto, Toronto, ON, Canada, ⁶ Department of Anaesthesiology, University of Toronto, Toronto, ON, Canada, ⁷ University Health Network, Toronto, ON, Canada, ⁸ Department of Human Physiology, University of Oregon, Eugene, Oregon, OR, USA, ⁹ Institute of Sports Sciences, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland, ¹⁰ Department of Physiology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland

OPEN ACCESS

Edited by:

Ovidiu Constantin Baltatu,
Camilo Castelo Branco University,
Brazil

Reviewed by:

Timo Partonen,
National Institute for Health and
Welfare, Finland
Karie Scrogin,
Loyola University Chicago, Stritch
School of Medicine, USA

*Correspondence:

Jui-Lin Fan
mickey.fan@otago.ac.nz

Specialty section:

This article was submitted to
Integrative Physiology,
a section of the journal
Frontiers in Physiology

Received: 02 October 2015

Accepted: 03 December 2015

Published: 08 January 2016

Citation:

Fan J-L, Subudhi AW, Duffin J,
Lovering AT, Roach RC and Kayser B
(2016) AltitudeOmics: Resetting of
Cerebrovascular CO₂ Reactivity
Following Acclimatization to High
Altitude. *Front. Physiol.* 6:394.
doi: 10.3389/fphys.2015.00394

Previous studies reported enhanced cerebrovascular CO₂ reactivity upon ascent to high altitude using linear models. However, there is evidence that this response may be sigmoidal in nature. Moreover, it was speculated that these changes at high altitude are mediated by alterations in acid-base buffering. Accordingly, we reanalyzed previously published data to assess middle cerebral blood flow velocity (MCAv) responses to modified rebreathing at sea level (SL), upon ascent (ALT1) and following 16 days of acclimatization (ALT16) to 5260 m in 21 lowlanders. Using sigmoid curve fitting of the MCAv responses to CO₂, we found the amplitude (95 vs. 129%, SL vs. ALT1, 95% confidence intervals (CI) [77, 112], [111, 145], respectively, $P = 0.024$) and the slope of the sigmoid response (4.5 vs. 7.5%/mmHg, SL vs. ALT1, 95% CIs [3.1, 5.9], [6.0, 9.0], respectively, $P = 0.026$) to be enhanced at ALT1, which persisted with acclimatization at ALT16 (amplitude: 177, 95% CI [139, 215], $P < 0.001$; slope: 10.3%/mmHg, 95% CI [8.2, 12.5], $P = 0.003$) compared to SL. Meanwhile, the sigmoidal response midpoint was unchanged at ALT1 (SL: 36.5 mmHg; ALT1: 35.4 mmHg, 95% CIs [34.0, 39.0], [33.1, 37.7], respectively, $P = 0.982$), while it was reduced by ~7 mmHg at ALT16 (28.6 mmHg, 95% CI [26.4, 30.8], $P = 0.001$ vs. SL), indicating leftward shift of the cerebrovascular CO₂ response to a lower arterial partial pressure of CO₂ (PaCO₂) following acclimatization to altitude. Sigmoid fitting revealed a leftward shift in the midpoint of the cerebrovascular response curve which could not be observed with linear fitting. These findings demonstrate that there is resetting of the cerebrovascular CO₂ reactivity operating point to a lower PaCO₂ following acclimatization to high altitude. This cerebrovascular resetting is likely the result of an altered acid-base buffer status resulting from prolonged exposure to the severe hypocapnia associated with ventilatory acclimatization to high altitude.

Keywords: cerebral blood flow, cerebral blood flow regulation, cerebral hemodynamics, high altitude, transcranial Doppler

INTRODUCTION

During metabolic and respiratory-mediated pH perturbations, both cerebral blood flow (CBF) and ventilation can be expressed as a single linear function of the hydrogen ion concentration ($[H^+]$) in extracellular fluid, and therefore cerebrospinal fluid (CSF) (Mitchell et al., 1965; Pappenheimer et al., 1965; Fencel et al., 1966, 1969; Skinhoj, 1966; Betz and Heuser, 1967; Severinghaus and Lassen, 1967; Lassen, 1968; Kontos et al., 1977; Warner et al., 1987; Smith et al., 1988; Toda et al., 1989). Since bicarbonate (HCO_3^-) is the main buffer base in the CSF compartment (Siesjö, 1972), changes in partial pressure of arterial carbon dioxide ($PaCO_2$) will lead to corresponding changes in $[H^+]$ unless compensatory adjustment in $[HCO_3^-]$ occurs (Mitchell et al., 1965). Accordingly, CSF $[HCO_3^-]$ is an important determinant of cerebrovascular and ventilatory responses during respiratory acid-base disturbances.

During prolonged exposure to hypoxia, the hyperventilation-induced hypocapnia and associated respiratory alkalosis lead to a compensatory renal HCO_3^- excretion, thereby lowering $[HCO_3^-]$ and returning pH toward homeostatic values in the arterial and CSF compartment (Dempsey et al., 1974, 1975, 1978; Forster et al., 1975). At sea-level, reducing CSF $[HCO_3^-]$ with oral ammonium chloride administration increases the cerebrovascular and ventilatory responsiveness to CO_2 , and elicits a resetting of these responses to lower $PaCO_2$ values (Fencel et al., 1969). This reduction in CSF $[HCO_3^-]$ and associated base buffering change leads to a greater increase in $[H^+]$ for a given rise in $PaCO_2$ (Siesjö, 1972). Such changes in acid-base buffering in the CSF compartment accounts for the enhanced CBF and ventilatory responsiveness to CO_2 observed at high altitude (Mathew et al., 1983; Schoene et al., 1990; Fan et al., 2014), along with the leftward shift in the ventilatory CO_2 sensitivity (Crawford and Severinghaus, 1978; Mathew et al., 1983; Fan et al., 2012, 2014). Pioneering work by Severinghaus (1965) estimated that CBF would be 78% above pre-altitude values if arterial partial pressures of O_2 (PaO_2) and CO_2 were returned to sea-level values following 3–5 days at 3800 m. Similarly, data from Fan et al. (2010, 2012) indicated a leftward shift in the CBF response to CO_2 following prolonged high altitude exposure. Since there is a delayed onset of compensatory metabolic acidification we would expect to observe no such resetting of the cerebrovascular CO_2 reactivity during acute high altitude exposure. However, no studies have identified the magnitude of this cerebrovascular resetting during acute and chronic exposure to high altitude.

Under constant arterial blood pressure, the overall relationship of $PaCO_2$ and CBF is sigmoidal with a linear portion between $PaCO_2$ of 25–65 mmHg (Madden, 1993). While the majority of the literature is focused on the linear portion of this response, sigmoid fitting might be more appropriate for the assessment of cerebrovascular CO_2 reactivity at high altitude due to the severe hypocapnia resulting from ventilatory acclimatization to high altitude. Further, the use of a linear model may miss a number of physiological parameters (e.g., the optimal operating point) and physical constraints of the cerebral vasculature (e.g. vessels' finite dilation/constriction and associated vascular reserves; Battisti-Charbonney et al., 2011).

Accordingly, sigmoid curve fitting could provide additional insight to the changes in cerebrovascular CO_2 reactivity at high altitude (such as changes in vascular dilation or constriction reserves and a shifting of the operating point).

Using the sigmoid curve fitting method described by Battisti-Charbonney et al. (2011), we reanalyzed our cerebrovascular CO_2 reactivity data from rebreathing tests as previously reported in Fan et al. (2014). We tested the hypotheses that: (1) ascent to high altitude is accompanied by a leftward shift as well as an increase in the cerebrovascular response to CO_2 upon ascent to 5260 m and following acclimatization; (2) these changes in cerebrovascular CO_2 reactivity would be abolished once we account for the acid-base change by plotting CBF against $[H^+]$ instead of PCO_2 ; and (3) the use of sigmoid fitting will reveal changes in vascular reserve at high altitude.

MATERIALS AND 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 >1500 m for more than 1 year or had traveled to altitudes >1000 m in the past 3 months. After obtaining written consent, physical exams and the Army Physical Fitness Test (push-ups, sit-ups, and a 3.2-km run) were performed to verify health and fitness status (Subudhi et al., 2014b).

Ethical Approval

The study was performed according to the *Declaration of Helsinki* and was approved by the Institutional Review Boards of the Universities of Colorado and 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 consent was given prior to participation.

Experimental Design

After familiarization with the experimental procedures outlined below (visit one), twenty-one subjects underwent three experimental trials: near sea level (SL: 130 m, barometric pressure: 749 mmHg) and on day 1 and 16 following ascent to 5260 m (ALT1 and ALT16). Approximately 4 weeks following SL measurements in Eugene, Oregon, the subjects were flown to La Paz, Bolivia. They spent two nights at low altitude (1525 m in Coroico, Bolivia) before being driven to the Chacaltaya Research Station at 5260 m, while breathing supplemental oxygen (ALT1). Subjects acclimatized to altitudes ranging from 3800 to 5260 m over the next 15 days, with most of the time (75%) spent at 5260 m. On the 16th day (ALT16), measurements were repeated (Subudhi et al., 2014b).

Experimental Protocol

A 22-gauge catheter was inserted into a radial artery at least 1 h prior to instrumentation. Subjects were seated in an upright

position for 15 min, while sensors were placed to measure physiological variables of interest (Subudhi et al., 2014b). Following 10–15 min of quiet rest in a seated position, each experimental testing session comprised of: (a) instrumentation; (b) 10 min room air baseline; (c) 10 min steady-state with partial pressure of end-tidal CO₂ (PETCO₂) and O₂ (PETO₂) clamped at 40 mmHg and 300 mmHg, respectively, and (d) assessment of cerebrovascular CO₂ reactivity with a modified rebreathing test. For each subject, all ALT measurements were carried out around the same time of day to minimize any confounding effect by circadian rhythm. Measurements were taken upon arrival at ALT1 to minimize the influence of acute mountain sickness (AMS). Likewise, no symptoms of AMS were observed at ALT16.

Throughout the protocol, the subjects sat upright and breathed through a mouthpiece attached to a two-way non-rebreathing valve (Hans-Rudolph 2700, Hans-Rudolph Inc., Shawnee, KS, USA). The breathing circuit allowed switching from room air to either an end-tidal clamping system or a rebreathing system.

Modified Rebreathing Method

The rebreathing bag was filled with gas to achieve inspired PCO₂ and PO₂ of 0 and 300 mmHg, respectively, upon rebreathing at each altitude. Hyperoxia was used to abolish the potentially confounding influence of changes in PaO₂ at SL, ALT1, and ALT16 on our cerebrovascular CO₂ reactivity estimates. Subjects were instructed to hyperventilate for 3 min to lower and then maintain PETCO₂ at ~18 mmHg at both sea level and 5260 m (in background PETO₂ >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*. The rebreathing tests were terminated when PETCO₂ reached 50 mmHg, PETO₂ dropped below 200 mmHg or the subject reached the end of his/her tolerance.

MEASUREMENTS

Cerebral Blood Flow Velocity

Middle cerebral artery velocity (MCAv, 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, USA). The Doppler ultrasound probe was positioned over the left temporal window and held in place with an adjustable plastic headband (Marc 600 Headframe, Spencer technology, Seattle, WA, USA). The signal was acquired at depths ranging from 43 to 54 mm. Signal quality was optimized and an M-mode screen shot was recorded to facilitate subsequent probe placements.

Arterial Blood Pressure

Beat-to-beat arterial blood pressure (ABP) 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, USA).

End-Tidal Gases

PETCO₂ and PETO₂ were measured using fast responding gas analyzers (O₂Cap Oxygen analyzer, Oxigraf, Mountain View, CA, USA). The gas analyzers were calibrated using gas mixtures of known concentrations of O₂ and CO₂ prior to each testing session.

Arterial Blood Gases

For each subject, arterial blood samples were taken four times at SL, ALT1 and ALT16 (room air breathing, PETCO₂ clamped at 40 mmHg, hyperventilation and end of rebreathing). Hydrogen ion concentration ([H⁺]) was calculated from the arterial pH values using the Siggaard-Andersen equation (OSA.exe, freely available at <http://www.siggaard-andersen.dk>). The [H⁺] values were plotted against PaCO₂ (Figure 1) and the relationship between these two variables was obtained using linear regression (Prism 6.0b, GraphPad Software Inc., San Diego, CA, USA). The equation of this [H⁺]-PaCO₂ slope was subsequently used to calculate the corresponding [H⁺] values for the range and the midpoint of the sigmoid curves for each rebreathing maneuver (Figure 1).

Data Acquisition

All analog data were sampled and recorded at 200 Hz on a PC for off-line analysis (ADInstruments Powerlab 16/30, Dunedin, New Zealand).

DATA ANALYSIS

Rebreathing Analysis (Figure 2)

As first detailed by Battisti-Charbonney et al. (2011), the following assumptions were made regarding the analysis of the rebreathing data: (i) hyperventilation lowers PETCO₂ sufficiently to produce full hypocapnia-induced vasoconstriction; (ii) as PETCO₂ increases from a hypocapnic value during hyperventilation to increasingly hypercapnic values during rebreathing, full vasodilation is achieved; the shape of this relationship is assumed to be sigmoidal with a linear portion

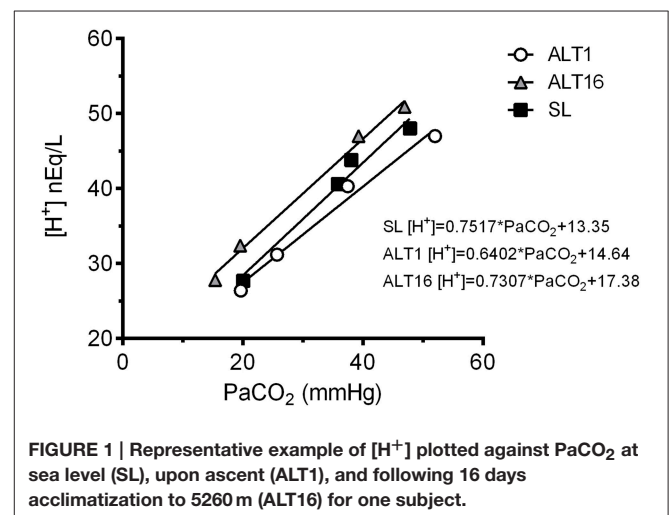


FIGURE 1 | Representative example of [H⁺] plotted against PaCO₂ at sea level (SL), upon ascent (ALT1), and following 16 days acclimatization to 5260 m (ALT16) for one subject.

in the middle of the curve (Claassen et al., 2007); and (iii) as hypercapnia increases beyond the point of maximal CO₂-induced vasodilation, CBF becomes directly dependent on perfusion pressure. Based on these assumptions, cerebrovascular CO₂ reactivity can be summarized as the vasodilatory and vasoconstrictive response of the cerebral blood vessels. The “midpoint” of the sigmoid curve then represents the optimal point of the vessels’ capacity to dilate and constrict (i.e., the optimal operating point), while the upper and lower plateaus of the sigmoid response represent the physical constraints of the cerebral vascular reserve. Meanwhile, the range of the sigmoid curve reflects the PCO₂ range of the linear portion of the sigmoid curve, and the amplitude represents the overall extent of this response.

The rebreathing data were first reduced to 1-s averages across the entire rebreathing period. MCAv values were then converted to a percentage change from MCAv values observed at resting PETCO₂. Since the ABP-CO₂ response is enhanced at high altitude (Ainslie and Burgess, 2008; Fan et al., 2014; Willie et al., 2015) and known to potentially confound estimates of cerebrovascular CO₂ reactivity (Betz, 1968; Regan et al., 2014), a PETCO₂ threshold (T_{ABP}) was first identified as the point from which mean ABP rose more than 0.5 mmHg per mmHg change in PETCO₂. This threshold was identified by fitting the mean ABP response to PETCO₂, with two straight lines above and below the T_{ABP} using linear regression, and restricting the slope of the lower portion to < 0.5 mmHg change in ABP per mmHg change in PETCO₂. The MCAv response to PETCO₂ was then divided into two portions below and above the T_{ABP} and the portion of MCAv-PETCO₂ response below T_{ABP} was fitted with a sigmoid curve as described by Battisti-Charbonney et al. (2011), thereby minimizing the confounding influence of ABP on the MCAv-CO₂ response. To minimize the sum of squares for non-linear regression (Levenberg-Marquardt algorithm) we used the equation:

$$\text{MCAv} = \text{minimum} + (\text{amplitude}/(1 + \exp(-(\text{PETCO}_2 - \text{midpoint})/\text{range})))$$

Where MCAv is the dependent variable in %, PETCO₂ is the independent variable in mmHg, *minimum* is the minimum MCAv determined from the mean MCAv of the hypocapnic (hyperventilation) region, *amplitude* is the amplitude of the response (i.e., the maximum MCAv value), *midpoint* is the PCO₂ value at the center of the sigmoid curve, and *range* is the PCO₂ range of the linear portion of the sigmoid (an inverse reflection of the slope of the linear portion). The slope of the sigmoid curve (i.e., cerebrovascular CO₂ reactivity) is subsequently calculated from the linear portion of the response. The vasoconstriction reserve was calculated as the difference between minimum MCAv and MCAv at resting PaCO₂, while the vasodilation reserve was calculated as the difference between the MCAv at T_{ABP} (or amplitude if no T_{ABP} was observed during rebreathing) and MCAv at resting PaCO₂, expressed at percentage MCAv (Figure 2).

The alignment and fitting processes were performed using custom written graphic analysis routines (LabVIEW, Nation

Instruments, Austin, TX, USA). The fitting program provided an r-square of the sigmoid and linear fits.

Statistical Analysis

Due to logistical impacts on planning and transportation, not all subjects were able to participate in all high-altitude studies; please see the Figures and Table for complete sample size reporting for each procedure. After calculating descriptive statistics [mean, 95% confidence interval (CI)], mixed model linear regression analysis (diagonal repeated covariance assumed) (IBM® SPSS® Statistics version 21, IBM® Corporation, Armonk, NY, USA) was used to evaluate the effects of altitude exposure and acclimatization (between SL, ALT1 and ALT16) on the midpoint, amplitude and range of the fitted sigmoid curves. Post hoc tests were performed on the midpoint, amplitude and range of the sigmoid curves between SL, ALT1 and ALT16 using the Holm-Sidak adjustment for multiple comparisons ($\alpha = 0.05$) (IBM® SPSS® Statistics version 21, IBM® Corporation, Armonk, NY, USA). Cohen’s *d*, as a measure of effect size, was calculated as $d = (M_1 - M_2)/SD$, where M_1 and M_2 are means of group 1 and 2; *SD* is standard deviations of pooled data. The effect size was considered negligible when $d < 0.2$, small when $d \geq 0.2$, moderate when $d \geq 0.5$, large when $d \geq 0.8$, and very large when $d \geq 1.3$ (Sullivan and Feinn, 2012). Results were considered significant at the alpha level <0.05. A priori power calculations ($\alpha = 0.05$, $\beta = 0.20$) were used to determine sample size and limit Type II error.

RESULTS

Detailed baseline characteristics of the 21 (nine women; age 21 ± 1 years) subjects participating in AltitudeOmics were presented elsewhere (Subudhi et al., 2014a). All 21 subjects performed the modified rebreathing test at SL and ALT16. However, 4 of the 21 subjects did not perform the modified rebreathing at ALT1. After excluding data due to unstable ABP during rebreathing, 13, 9, and 7 trials met the inclusion criteria at SL, ALT1, and ALT16, respectively, and were included in the analysis. Sigmoid curve

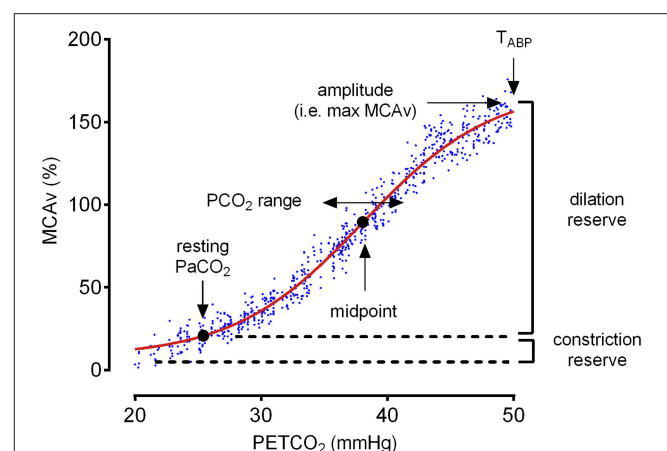


FIGURE 2 | A representative example of an individual rebreathing test and sigmoid curve fitting upon ascent to 5260 m (ALT1).

fitting was applied to the remaining trials with R^2 of 0.89 ± 0.05 (mean \pm SD).

Cerebrovascular CO₂ Reactivity

No difference was observed in the PETCO₂ range of the linear portion of the sigmoid between SL and ALT1 ($d = 1.0$, $P = 0.149$). Likewise, the PETCO₂ range was unaltered following acclimatization at ALT16 when compared to SL ($d = 1.2$, $P = 0.060$) or to ALT1 ($d = 0.3$, $P = 0.149$ vs. ALT1, **Table 1**). Upon ascent to 5260 m (ALT1), the amplitude of the MCAv response was increased by $\sim 34\%$ ($d = 5.8$, $P = 0.024$ vs. SL), and further increased by $\sim 82\%$ at ALT16 ($d = 11.1$, $P < 0.001$ vs. SL, **Table 1**). However, the amplitude of the sigmoid MCAv response was not different between ALT1 and ALT16 ($d = 7.3$, $P = 0.101$, **Table 1**). As a result of these increases in amplitude of the MCAv response, the linear portion of the sigmoid curve was enhanced by $\sim 67\%$ at ALT1 ($d = 1.8$, $P = 0.026$) and by $\sim 129\%$ at ALT16 ($d = 3.0$, $P = 0.003$) compared to SL, while no difference was observed from ALT1 to ALT16 ($d = 1.7$, $P = 0.175$, **Table 1**). The PETCO₂ midpoint of the sigmoid curve was unchanged upon ascent to 5260 m ($d = 0.5$, $P = 0.982$ vs. SL), while it was reduced by ~ 8 mmHg (i.e., shifted leftward) following acclimatization at ALT16 ($d = 3.3$, $P = 0.001$ vs. SL, **Table 1**). Accordingly, the PETCO₂ midpoint was lower at ALT16 by ~ 7 mmHg compared to initial exposure to high altitude ($d = 3.1$, $P = 0.005$ vs. ALT1, **Table 1**). These findings indicate that cerebrovascular CO₂ reactivity was enhanced upon ascent to 5260 m, resulting in both upward shifting and steepening of the sigmoid curve compared to sea level (**Figure 3**). Following acclimatization, at ALT16, there was a leftward shift of the sigmoid curve (**Figure 4A**), while, cerebrovascular CO₂ reactivity did not differ greatly between ALT1 and ALT16 (**Figure 3**).

Compared to SL, the vasoconstriction reserve was lowered by $\sim 31\%$ at both ALT1 ($d = 1.2$, $P = 0.008$) and at ALT16 ($d = 1.2$, $P = 0.012$), while the vasodilation reserve was increased by $\sim 83\%$ at ALT1 ($d = 1.67$, $P < 0.001$) and by $\sim 92\%$ at ALT16 ($d = 1.7$, $P < 0.001$, **Table 1**). No differences were

observed between ALT1 and ALT16 for the constriction ($d = 0.0$, $P = 1.000$) and dilation reserves ($d = 0.2$, $P = 0.945$, **Table 1**).

Acid-Base Buffering

When expressing MCAv against $[H^+]$, the range was unaltered following ascent at ALT1 ($d = 1.0$, $P = 0.137$ vs. SL) or with acclimatization at ALT16 ($d = 0.9$, $P = 0.171$), and no difference in the $[H^+]$ range of the linear sigmoid portion was observed between ALT1 and ALT16 ($d = 0.2$, $P = 0.993$, **Table 1**). Similarly, the midpoint of the sigmoid curve occurred at similar $[H^+]$ at SL, ALT1 ($d = 0.0$, $P = 1.000$ vs. SL) and ALT16 ($d = 0.1$, $P = 0.999$, **Table 1**). Meanwhile, cerebrovascular CO₂ reactivity was enhanced upon ascent to ALT1 by $\sim 78\%$ ($d = 2.4$, $P = 0.012$ vs. SL) and remained elevated at ALT16 ($d = 3.4$, $P = 0.004$, **Table 1**). No difference was found in cerebrovascular CO₂ reactivity between ALT1 and ALT16 ($d = 1.4$, $P = 0.396$, **Table 1**).

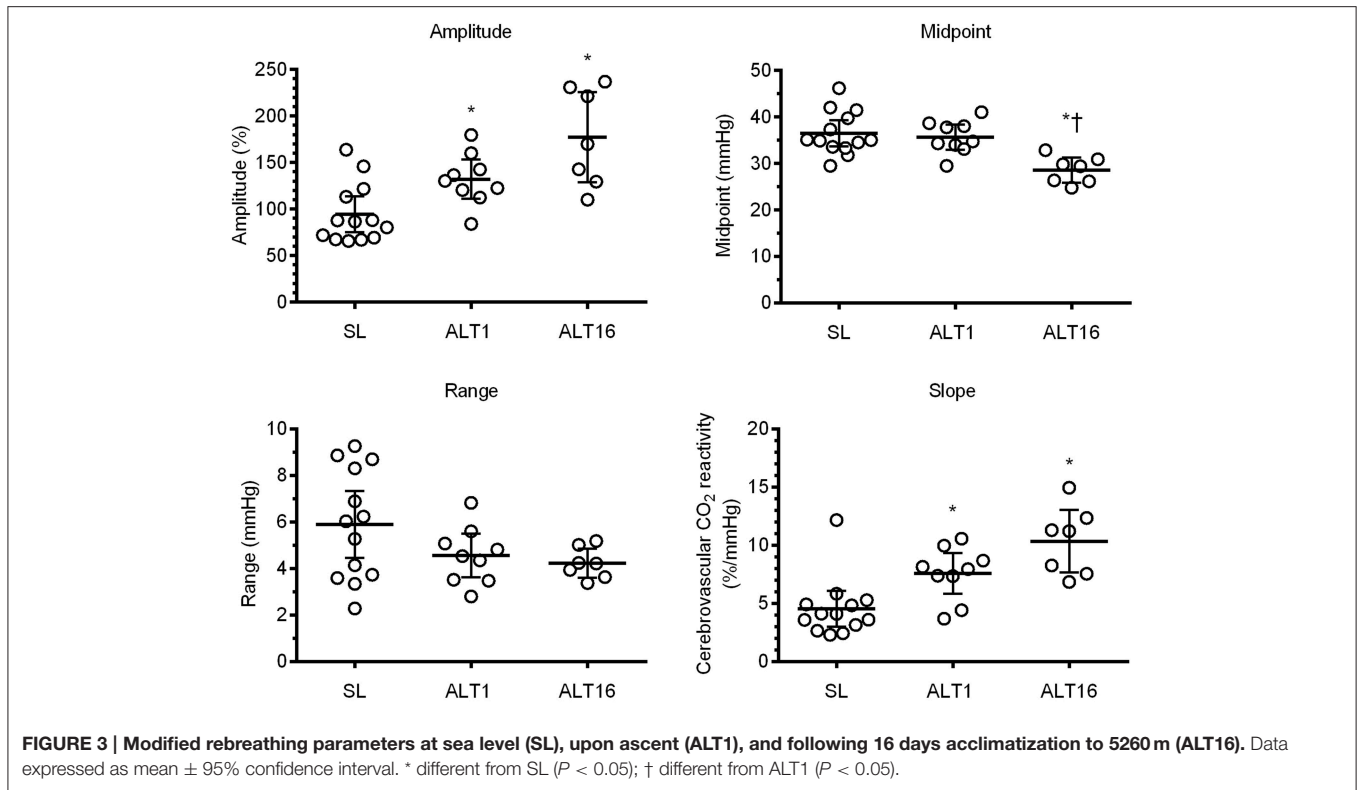
DISCUSSION

In the present study, we examined the changes in cerebrovascular parameters during modified rebreathing using sigmoid curve fitting analysis. Using sigmoid fitting, we found: (1) the increase in cerebrovascular CO₂ reactivity at ALT1 is mediated by an enhanced sensitivity of the cerebral blood vessels to H^+ (**Figure 4B**); (2) there is a leftward shift of MCAv-CO₂ response with acclimatization at ALT16 (**Figure 4A**), indicating a resetting of the cerebrovascular CO₂ reactivity sigmoid midpoint to a lower PaCO₂ with chronic hypoxia; (3) this leftward shift in cerebrovascular CO₂ reactivity is abolished once we account for the changed acid-base buffering status; and 4) despite this cerebrovascular resetting, the vascular constriction reserve is reduced at high altitude. From our findings, it appears that this cerebrovascular resetting to a lower range of PaCO₂, mediated by changes in acid-base buffering, is likely a serendipitous consequence of acclimatization to high altitude. Importantly, this resetting restores cerebrovascular control during the persistent

TABLE 1 | Sigmoid curve fitting parameters during modified rebreathing.

Variable <i>n</i>	SL 13		ALT1 9		ALT16 7	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Amplitude (%)	95	[77–112]	129*	[111–147]	177*	[139–216]
Midpoint (mmHg)	36.5	[34.0–39.0]	35.4	[33.1–37.7]	28.6††	[26.4–30.8]
Range (mmHg)	5.9	[4.6–7.2]	4.5	[3.7–5.3]	4.2	[3.7–4.7]
Cerebrovascular CO ₂ reactivity (%/mmHg)	4.5	[3.1–5.9]	7.5*	[6.0–9.0]	10.3*	[8.2–12.5]
Constriction reserve (%)	56	[42–70]	25*	[16–34]	25*	[15–36]
Dilation reserve (%)	20	[7–34]	103*	[82–125]	112*	[84–139]
PLOTTED AGAINST $[H^+]$						
Midpoint (nEq/L)	37.5	[35.0–40.1]	37.7	[36.7–38.7]	37.4	[35.3–39.4]
Range (nEq/L)	4.2	[3.3–5.1]	2.9	[2.4–3.4]	3.1	[2.6–3.6]
Cerebrovascular CO ₂ reactivity (%/nEq/L)	6.4	[4.5–8.4]	11.4*	[9.3–13.5]	14.1*	[11.2–17.1]

Different from SL (*) and ALT1 (†), $P < 0.05$.



severe hypocapnia associated with prolonged exposure to high altitude.

CBF regulation during both acute and chronic hypoxia can be summarized as the *net effect* of hypoxia-induced vasodilation and hypocapnia-induced vasoconstriction of the cerebral blood vessels (Xu and Lamanna, 2006; Brugniaux et al., 2007). During initial exposure to severe hypoxia, the vasodilatory drive in the cerebral blood vessels typically exceeds that of the constrictor drive, thereby elevating CBF (Severinghaus et al., 1966; Subudhi et al., 2014c). During acclimatization, the progressive increase in ventilatory drive lowers PaCO₂ and elevates PaO₂ (Brugniaux et al., 2007), resulting in greater vasoconstrictor drive and restoring of CBF to pre-exposure values (Severinghaus et al., 1966; Subudhi et al., 2014c). In the absence of a cerebrovascular resetting, the progressive increase in ventilatory drive and associated hyperventilation-induced hypocapnia with acclimatization would eventually lead to cerebral hypoperfusion. Therefore, the observed resetting of cerebrovascular CO₂ reactivity to a lower PCO₂ operating point mitigates the adverse effects of hypocapnia-induced vasoconstriction, thus enabling the cerebral vessels to better regulate CBF during prolonged high altitude exposure.

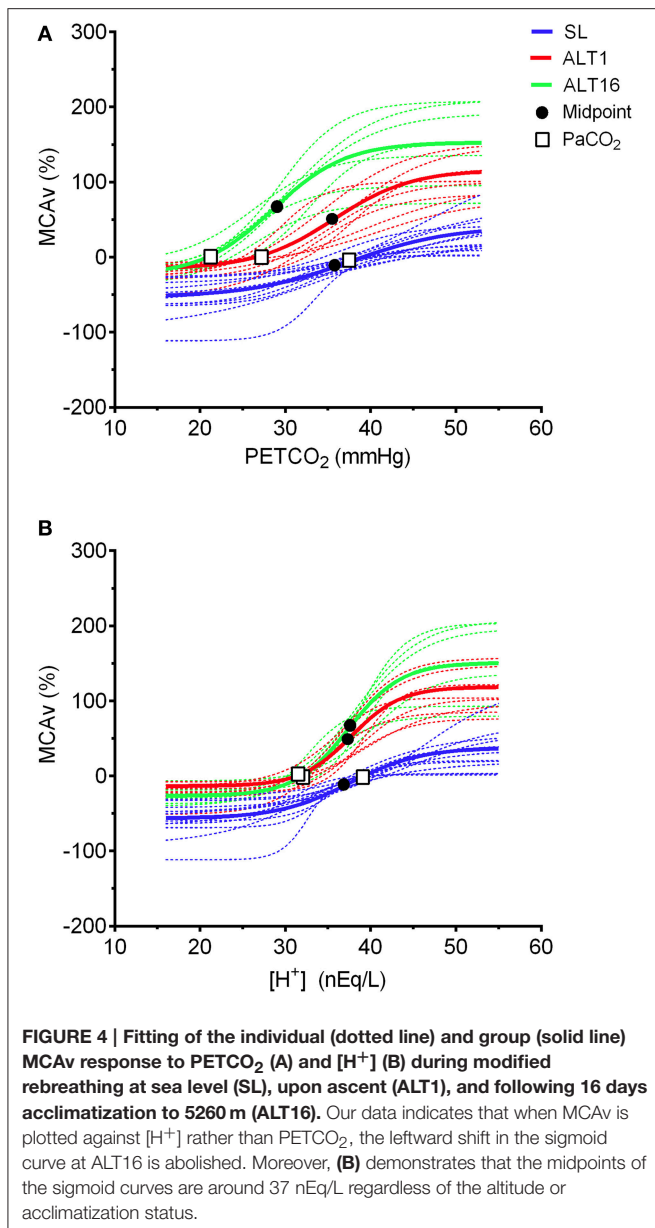
Altered Acid-Base Buffering

The CSF is a bicarbonate-containing fluid which acts as an important acid-base buffer in the regulation in CSF pH (Siesjö, 1972). Since there are negligible concentrations of protein and other non-bicarbonate buffers in the CSF (Davson, 1967), changes in [HCO₃⁻] reflect changes in buffer base (Siesjö,

1972). As elegantly summarized by Fencl et al. (1969), the regulation of CBF and ventilation are components of the same homeostatic system, where the input consists of [H⁺] in the CSF compartment, which is determined by [HCO₃⁻] and PCO₂. In this system, the respiratory loop regulates PaCO₂ (and therefore [H⁺] controlling CBF), while the cerebrovascular loop regulates the PCO₂ gradient between arterial and CSF compartment (and therefore [H⁺] at the level of the central chemoreceptors). Importantly, the bicarbonate concentration provides the link between the two regulatory loops of this system. Under conditions of metabolic acidosis or alkalosis, CSF [HCO₃⁻] is regulated to reduce CSF pH variations to one tenth of that occurring in the arterial compartment (Fencl et al., 1969; Dempsey et al., 1978). Since a CSF pH change of 0.2 unit covers the entire ventilatory response from life-threatening hypoventilation to maximal hyperventilation (Pappenheimer, 1967), a close regulation of CSF pH is critical for normal respiratory function. As CSF [HCO₃⁻] is an important link between cerebrovascular and ventilatory control, any bicarbonate-mediated changes in ventilatory CO₂ sensitivity should be reflected in cerebrovascular CO₂ reactivity. Moreover, changes in CSF [HCO₃⁻] would lead to proportional changes in the PCO₂ range within which CBF and ventilation are regulated. In support, we found there was a leftward shift of the PaCO₂-[H⁺] relationship at ALT16 (Table 1).

Enhanced Sensitivity to H⁺ during Acute Exposure

During respiratory alkalosis, as observed during hypoxic exposure, a reduced CSF [HCO₃⁻] leads to a greater increase



in [H⁺] for a given rise in PaCO₂ (Siesjö, 1972). Previously, we speculated these changes in acid-base buffering following prolonged hypoxic exposure could account for the enhanced cerebrovascular CO₂ reactivity during initial exposure to high altitude (Fan et al., 2010). Accordingly, any increase in cerebrovascular CO₂ reactivity at high altitude should be abolished when plotting MCAv changes against [H⁺] instead of PCO₂. However, in the present analysis we found the increase in sigmoid slope at ALT1 persisted when we plotted MCAv against [H⁺] (Figure 4B), which indicates that this increase in cerebrovascular CO₂ reactivity upon ascent to high altitude is mediated by an enhanced H⁺ sensitivity in the cerebral vasculature *per se*, rather than altered acid-base balance only.

The presence of a cerebrovascular function resetting at high altitude was first alluded to by Severinghaus (1965) and later by Fan et al. (2010). In support, early work by Fencl et al. (1969) reported leftward shifts in both cerebrovascular and ventilation responses to CO₂ following a 5 day administration of sodium bicarbonate (inducing metabolic alkalosis), while chronic metabolic acidosis with ammonium chloride elicited an opposite effect on these CO₂ responses. High altitude studies have reported a leftward shift in the ventilatory response to CO₂ (Crawford and Severinghaus, 1978; Mathew et al., 1983; Somogyi et al., 2005; Ainslie and Burgess, 2008; Fan et al., 2010), which can be predicted by modeling the acid-base changes in the CSF compartment (Duffin, 2005). Using CO₂ supplementation, Cruz et al. (1980) demonstrated that the leftward shift in the ventilatory response to CO₂ observed following 75 h of altitude exposure can be abolished by preventing the respiratory alkalosis (and subsequent negative base excess). These findings suggest that the changes in acid-base balance are responsible for the resetting of resting CBF and the leftward shifts in both cerebrovascular and ventilatory responses to CO₂ during acclimatization to high altitude. In the present study, the leftward shift in the cerebrovascular CO₂ reactivity at ALT16 was abolished when the MCAv changes were plotted against [H⁺] instead of PETCO₂ (Figure 4). As a result, the midpoints of the sigmoid curves appeared at similar [H⁺] (~37 nEq/L) across all three time-points (Figure 4B). From this, we speculate that the observed leftward shift of the sigmoid curve at ALT16 is likely a consequence of reduced [HCO₃⁻] in the CSF, resulting from the prolonged exposure to hypoxia-induced hypocapnia. Moreover, our findings indicate that the cerebral reserves for vasoconstriction and vasodilation are most optimal around 37 nEq/L H⁺ concentration, regardless of altitude or acclimatization status. Our data support the notion that H⁺-mediated cerebrovascular control is highly conserved.

Altered Cerebrovascular Reserves at High Altitude

The midpoint of the sigmoid cerebrovascular reactivity curve represents the optimization point between maximal vasoconstriction and vasodilation (Battisti-Charbonney et al., 2011). A departure of resting PaCO₂ from the midpoint toward the lower plateau lowers the vascular reserve for further constriction, while increasing PaCO₂ toward the upper plateau lowers the vascular reserve for dilation (Figure 4A). In addition, outside the linear portion of the sigmoid curve, the CBF responsiveness to changes in CO₂ is blunted. Together these changes in PaCO₂ and associated reductions in vascular reserve for constriction and dilation limit the blood vessels' ability to regulate CBF against perturbations in either PaCO₂ or ABP. As reported in our previous study (Subudhi et al., 2014a), hyperventilation at 5260 m lowered resting PaCO₂ by ~12 mmHg at ALT1 and by ~17 mmHg at ALT16. These reductions in resting PaCO₂ lowered the vascular constriction reserve by ~30% upon ascent to 5260 m and following acclimatization, despite the compensatory leftward shift in the cerebrovascular CO₂ reactivity curve at ALT16 (Figure 4A).

Our present findings indicate that hyperventilation-induced hypocapnia reduced the vascular reserve for vasoconstriction at high altitude, thereby blunting the vessel's ability to respond to changes in CO₂ (and presumably blood pressure) at 5260 m. Importantly, this reduced vascular reserve and associated blunting of the cerebral vessels' responsiveness could potentially account for the impaired cerebral autoregulation observed during acute and chronic exposure to hypoxia, as previously reported by Subudhi et al. (2014b) and others (Jansen et al., 2000; Ainslie et al., 2008; Iwasaki et al., 2011).

Confounding Influence of Background Hypoxia and Blood Pressure

The effect of high altitude exposure on cerebrovascular CO₂ reactivity has been examined by a number of studies (Jensen et al., 1996; Jansen et al., 1999; Ainslie and Burgess, 2008; Fan et al., 2010, 2012, 2014; Lucas et al., 2011; Villien et al., 2013; Rupp et al., 2014; Flück et al., 2015; Willie et al., 2015). More recently, studies have assessed cerebrovascular CO₂ reactivity in *background hypoxia* (Rupp et al., 2014; Flück et al., 2015; Willie et al., 2015), which has been shown to blunt the CBF response to CO₂ (McPherson et al., 1987; Fan et al., 2013; Ogoh et al., 2014)—presumably by exhausting the dilatory response and reducing prostanoid synthesis (Leffler et al., 1986). Those studies have found cerebrovascular CO₂ reactivity to be either reduced (Rupp et al., 2014; Flück et al., 2015), or unchanged (Willie et al., 2015) following ascent to high altitude, while ventilatory responsiveness to CO₂ was enhanced. In contrast, studies assessing cerebrovascular CO₂ reactivity in background hyperoxia have consistently found it to be elevated with high altitude ascent (Fan et al., 2010, 2012, 2014) and prolonged exposure to hypoxia (Poulin et al., 2002), coinciding with enhanced ventilatory CO₂ sensitivity. Due to the confounding effects of hypoxia, the cerebrovascular responses to CO₂ assessed in *background hypoxia* may not necessarily reflect true cerebrovascular CO₂ reactivity *per se* and thus should be interpreted with caution.

In agreement with our previous report using linear fitting (Fan et al., 2014), we found cerebrovascular CO₂ reactivity to be enhanced by 85% upon ascent at ALT1, while no further increase was observed following the acclimatization period in this study (Table 1). Similar to other high altitude studies (Flück et al., 2015; Willie et al., 2015), we observed large elevations in blood pressure during CO₂ breathing (Fan et al., 2014). Since increases in systemic blood pressure influence the CBF response to CO₂ (Betz, 1968) and thus confounds the measurement of cerebrovascular CO₂ reactivity (Regan et al., 2014), we attribute this smaller additional increase in cerebrovascular CO₂ reactivity at ALT16 to the constant ABP during the rebreathing trials analyzed in this study. Therefore, it should be acknowledged that the previously reported changes of cerebrovascular CO₂ reactivity at high altitude reflect the CBF response to the combined effects of both CO₂ and arterial blood pressure, rather than CO₂ alone. However, we cannot exclude the possibility that the lack of increase

at ALT16 compared to ALT1 may be due a lower subject number.

Limitations

One of the critiques when using TCD-measured MCAv as an index of CBF is the assumption that middle cerebral artery (MCA) diameter does not change. Due to the exponential dependency of the vessel cross-sectional area on the diameter, small changes in MCA diameter would result in considerable errors in CBF estimates with TCD. As mentioned in our previous study (Fan et al., 2014), we assessed MCAv changes in a background of hyperoxia (PETO₂ >250 mmHg), thus excluding any confounding dilatory effects of hypoxia on MCA diameter in our measurements. While early magnetic resonance imaging (MRI) studies found no changes in MCA diameter during changes in PETCO₂ between 24 and 45 mmHg (Valdúeza et al., 1997; Serrador et al., 2000), more recent and higher resolution MRI studies reported changes in MCA diameter (1.2–8%) during voluntary hyperventilation (PETCO₂: 27–24 mmHg) and hypercapnia (PETCO₂: 45–51 mmHg) (Coverdale et al., 2014; Verbree et al., 2014). The major implications of these MCA diameter changes are *overestimation* of CBF at low PaCO₂, and *underestimation* of CBF at high PaCO₂ with TCD. In agreement, Willie et al. (2015) found TCD to underestimate cerebrovascular CO₂ reactivity when compared to the vascular Doppler ultrasound method. However, we contend that our assessment of CBF with TCD estimates are reliable since: (i) Caputi et al. (2014) have shown similar values of cerebrovascular CO₂ reactivity using MRI and TCD estimates of CBF response to 5% CO₂ breathing; and (ii) all the temporal indices such as the midpoint and range of the sigmoid curve would be unaffected by any underestimation of CBF with TCD. Another important consideration when interpreting our findings is our use of arterial pH and acid-base buffering as a surrogate for CSF pH changes at high altitude. Dempsey et al. (1974) previously reported consistent pH differences (~0.08), and similar [HCO₃⁻] between arterial and lumbar spinal fluid in seven subjects at sea-level, and following 1, 8 h, and 3–4 weeks at 3100 m. Moreover, they found identical pH compensation (66%) in both arterial and lumbar spinal fluid compartments following acclimatization to high altitude. The data from Dempsey et al. (1974) thus support the use of arterial acid-base changes as an index of CSF changes during acute and chronic exposure to high altitude.

CONCLUSION

The present study is the first to demonstrate a remodeling of the sigmoid cerebrovascular CO₂ reactivity curve following acclimatization to high altitude. Our data indicates that the increase in cerebrovascular CO₂ reactivity upon ascent to high altitude is mediated by enhanced H⁺ sensitivity of the cerebral vasculature. Meanwhile, acclimatization to 5260 m leads to a leftward shift in cerebrovascular CO₂ reactivity which appears to be mediated by an altered acid-base buffering following prolonged exposure to hypoxia. We speculate that such cerebrovascular resetting is critical for maintaining normal

cerebrovascular control and preventing cerebral hypoperfusion from the prolonged severe hypocapnia associated with high altitude acclimatization.

AUTHOR CONTRIBUTIONS

JF, AS, BK, AL, and RR contributed to conception and design of the experiments. JF and AS performed the experiments. JF, AS, and JD analysed the data. JF, AS, JD, BK, and RR interpreted the results of the experiments. JF, AS, and JD prepared the figures and drafted the manuscript. JF, AS, JD, BK, AL, and RR edited and revised the manuscript. JF, AS, JD, BK, AL, and RR approved the final version of the manuscript.

FUNDING

This study was supported by the Swiss National Science Foundation and the Faculty of Medicine of the University of Geneva. The overall AltitudeOmics study was funded, in part, by grants from the U.S. Department of Defense (W81XWH-11-2-0040 TATRC to RR, and W81XWH-10-2-0114 to AL); the Cardiopulmonary and Respiratory Physiology Laboratory, University of Oregon; the Altitude Research Center and the Charles S. Houston Endowed Professorship, Department

REFERENCES

- Ainslie, P. N., and Burgess, K. R. (2008). Cardiorespiratory and cerebrovascular responses to hyperoxic and hypoxic rebreathing: effects of acclimatization to high altitude. *Respir. Physiol. Neurobiol.* 161, 201–209. doi: 10.1016/j.resp.2008.02.003
- Ainslie, P. N., Ogoh, S., Burgess, K., Celi, L., McGrattan, K., Peebles, K., et al. (2008). Differential effects of acute hypoxia and high altitude on cerebral blood flow velocity and dynamic cerebral autoregulation: alterations with hyperoxia. *J. Appl. Physiol.* 104, 490–498. doi: 10.1152/jappphysiol.00778.2007
- Battisti-Charbonney, A., Fisher, J., and Duffin, J. (2011). The cerebrovascular response to carbon dioxide in humans. *J. Physiol.* 589, 3039–3048. doi: 10.1113/jphysiol.2011.206052
- Betz, E. (1968). The significance of cortical extracellular pH for the regulation of blood flow in the cerebral cortex. *Prog. Brain Res.* 30, 99–102. doi: 10.1016/S0079-6123(08)61445-4
- Betz, E., and Heuser, D. (1967). Cerebral cortical blood flow during changes of acid-base equilibrium of the brain. *J. Appl. Physiol.* 23, 726–733.
- Brugniaux, J. V., Hodges, A. N., Hanly, P. J., and Poulin, M. J. (2007). Cerebrovascular responses to altitude. *Respir. Physiol. Neurobiol.* 158, 212–223. doi: 10.1016/j.resp.2007.04.008
- Caputi, L., Ghielmetti, F., Farago, G., Longaretti, F., Lamperti, M., Anzola, G. P., et al. (2014). Cerebrovascular reactivity by quantitative magnetic resonance angiography with a Co(2) challenge. Validation as a new imaging biomarker. *Eur. J. Radiol.* 83, 1005–1010. doi: 10.1016/j.ejrad.2014.03.001
- Claassen, J. A., Zhang, R., Fu, Q., Witkowski, S., and Levine, B. D. (2007). Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. *J. Appl. Physiol.* 102, 870–877. doi: 10.1152/jappphysiol.00906.2006
- Coverdale, N. S., Gati, J. S., Opalevych, O., Perrotta, A., and Shoemaker, J. K. (2014). Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. *J. Appl. Physiol.* (1985) 117, 1090–1096. doi: 10.1152/jappphysiol.00285.2014
- Crawford, R. D., and Severinghaus, J. W. (1978). CSF pH and ventilatory acclimatization to altitude. *J. Appl. Physiol.* 45, 275–283.
- Cruz, J. C., Reeves, J. T., Grover, R. F., Maher, J. T., McCullough, R. E., Cymerman, A., et al. (1980). Ventilatory acclimatization to high altitude is prevented by CO₂ breathing. *Respiration* 39, 121–130. doi: 10.1159/000194206

of Emergency Medicine, School of Medicine, University of Colorado Denver.

ACKNOWLEDGMENTS

This paper is part of a series titled “AltitudeOmics” that together represent a group of studies that explored the basic mechanisms controlling human acclimatization to hypoxia and its subsequent retention. Many people and organizations invested enormous amounts of time and resources to make AltitudeOmics a success. Foremost, the study was made possible by the tireless support, generosity and tenacity of our research subjects. AltitudeOmics principal investigators were Colleen G. Julian, AL, AS, and RR. A complete list of other investigators on this multinational, collaborative effort involved in development, subject management and data collection, supporting industry partners, and people and organizations in Bolivia that made AltitudeOmics possible is available in the first paper in this series (Subudhi et al., 2014a). The authors are extremely grateful to Julia Kern, Oghenero Evero, Jon E. Elliot, Steve S. Laurie, Kara M. Beasley and Nicolas Bourdillon for their invaluable assistance in the data collection for this study. We would like to extend our thanks to Roberto Molinari for his technical assistance in the statistical analysis of the data.

- Davson, H. (1967). *Physiology of the Cerebrospinal Fluid*. London: J and A Churchill.
- Dempsey, J. A., Forster, H. V., Chosy, L. W., Hanson, P. G., and Reddan, W. G. (1978). Regulation of CSF[HCO₃⁻] during long-term hypoxic hypocapnia in man. *J. Appl. Physiol.* 44, 175–182.
- Dempsey, J. A., Forster, H. V., and Dopico, G. A. (1974). Ventilatory acclimatization to moderate hypoxemia in man: the role of spinal fluid [H⁺]. *J. Clin. Invest.* 53, 1091–1100. doi: 10.1172/JCI107646
- Dempsey, J. A., Forster, H. V., Gledhill, N., and Dopico, G. A. (1975). Effects of moderate hypoxemia and hypocapnia on CSF [H⁺] and ventilation in man. *J. Appl. Physiol.* 38, 665–674.
- Duffin, J. (2005). Role of acid-base balance in the chemoreflex control of breathing. *J. Appl. Physiol.* 99, 2255–2265. doi: 10.1152/jappphysiol.0064.0.2005
- Fan, J. L., Bourdillon, N., and Kayser, B. (2013). Effect of end-tidal CO₂ clamping on cerebrovascular function, oxygenation, and performance during 15-km time trial cycling in severe normobaric hypoxia: the role of cerebral O₂ delivery. *Physiol. Rep.* 1:e00066. doi: 10.1002/phy2.66
- Fan, J. L., Burgess, K. R., Basnyat, R., Thomas, K. N., Peebles, K. C., Lucas, S. J., et al. (2010). Influence of high altitude on cerebrovascular and ventilatory responsiveness to CO₂. *J. Physiol.* 588, 539–549. doi: 10.1113/jphysiol.2009.184051
- Fan, J. L., Burgess, K. R., Thomas, K. N., Lucas, S. J., Cotter, J. D., Kayser, B., et al. (2012). Effects of acetazolamide on cerebrovascular function and breathing stability at 5050 m. *J. Physiol.* 590, 1213–1225. doi: 10.1113/jphysiol.2011.219923
- Fan, J. L., Subudhi, A. W., Evero, O., Bourdillon, N., Kayser, B., Lovering, A. T., et al. (2014). AltitudeOmics: enhanced cerebrovascular reactivity and ventilatory response to CO₂ with high-altitude acclimatization and reexposure. *J. Appl. Physiol.* (1985) 116, 911–918. doi: 10.1152/jappphysiol.00704.2013
- Fencel, V., Miller, T. B., and Pappenheimer, J. R. (1966). Studies on the respiratory response to disturbances of acid-base balance, with deductions concerning the ionic composition of cerebral interstitial fluid. *Am. J. Physiol.* 210, 459–472.
- Fencel, V., Vale, J. R., and Broch, J. A. (1969). Respiration and cerebral blood flow in metabolic acidosis and alkalosis in humans. *J. Appl. Physiol.* 27, 67–76.
- Flück, D., Siebenmann, C., Keiser, S., Cathomen, A., and Lundby, C. (2015). Cerebrovascular reactivity is increased with acclimatization to 3,454 m altitude. *J. Cereb. Blood Flow Metab.* 35, 1323–1330. doi: 10.1038/jcbfm.2015.51

- Forster, H. V., Dempsey, J. A., and Chosy, L. W. (1975). Incomplete compensation of CSF [H⁺] in man during acclimatization to high altitude (48300 M). *J. Appl. Physiol.* 38, 1067–1072.
- Iwasaki, K., Zhang, R., Zuckerman, J. H., Ogawa, Y., Hansen, L. H., and Levine, B. D. (2011). Impaired dynamic cerebral autoregulation at extreme high altitude even after acclimatization. *J. Cereb. Blood Flow Metab.* 31, 283–292. doi: 10.1038/jcbfm.2010.88
- Jansen, G. F., Krins, A., and Basnyat, B. (1999). Cerebral vasomotor reactivity at high altitude in humans. *J. Appl. Physiol.* 86, 681–686.
- Jansen, G. F., Krins, A., Basnyat, B., Bosch, A., and Odoom, J. A. (2000). Cerebral autoregulation in subjects adapted and not adapted to high altitude. *Stroke* 31, 2314–2318. doi: 10.1161/01.STR.31.10.2314
- Jensen, J. B., Sperling, B., Severinghaus, J. W., and Lassen, N. A. (1996). Augmented hypoxic cerebral vasodilation in men during 5 days at 3,810 m altitude. *J. Appl. Physiol.* 80, 1214–1218.
- Kontos, H. A., Raper, A. J., and Patterson, J. L. (1977). Analysis of vasoactivity of local pH, PCO₂ and bicarbonate on pial vessels. *Stroke* 8, 358–360. doi: 10.1161/01.STR.8.3.358
- Lassen, N. A. (1968). Brain extracellular pH: the main factor controlling cerebral blood flow. *Scand. J. Clin. Lab. Invest.* 22, 247–251. doi: 10.3109/00365516809167060
- Leffler, C. W., Busija, D. W., Beasley, D. G., Fletcher, A. M., and Green, R. S. (1986). Effects of indomethacin on cardiac output distribution in normal and asphyxiated piglets. *Prostaglandins* 31, 183–190. doi: 10.1016/0090-6980(86)90045-6
- Lucas, S. J. E., Burgess, K. R., Thomas, K. N., Donnelly, J., Peebles, K. C., Lucas, R. A. I., et al. (2011). Alterations in cerebral blood flow and cerebrovascular reactivity during 14 days at 5050 m. *J. Physiol.* 589, 741–753. doi: 10.1113/jphysiol.2010.192534
- Madden, J. A. (1993). The effect of carbon dioxide on cerebral arteries. *Pharmacol. Ther.* 59, 229–250. doi: 10.1016/0163-7258(93)90045-F
- Mathew, L., Gopinath, P. M., Purkayastha, S. S., Sen Gupta, J., and Nayar, H. S. (1983). Chemoreceptor sensitivity in adaptation to high altitude. *Aviat. Space Environ. Med.* 54, 121–126.
- McPherson, R. W., Eimerl, D., and Traystman, R. J. (1987). Interaction of hypoxia and hypercapnia on cerebral hemodynamics and brain electrical activity in dogs. *Am. J. Physiol.* 253, H890–H897.
- Mitchell, R. A., Carman, C. T., Severinghaus, J. W., Richardson, B. W., Singer, M. M., and Shnider, S. (1965). Stability of cerebrospinal fluid pH in chronic acid-base disturbances in blood. *J. Appl. Physiol.* 20, 443–452.
- Ogoh, S., Nakahara, H., Ueda, S., Okazaki, K., Shibasaki, M., Subudhi, A. W., et al. (2014). Effects of acute hypoxia on cerebrovascular responses to carbon dioxide. *Exp. Physiol.* 99, 849–858. doi: 10.1113/expphysiol.2013.076802
- Pappenheimer, J. R. (1967). “The ionic composition of cerebral extracellular fluid and its relation to control of breathing,” in *The Harvey Lectures 1965–1966*. (New York, NY; London: Academic Press), 71.
- Pappenheimer, J. R., Fencl, V., Heisey, S. R., and Held, D. (1965). Role of cerebral fluids in control of respiration as studied in unanesthetized goats. *Am. J. Physiol.* 208, 436–450.
- Poulin, M. J., Fatemian, M., Tansley, J. G., O’connor, D. F., and Robbins, P. A. (2002). Changes in cerebral blood flow during and after 48 h of both isocapnic and poikilocapnic hypoxia in humans. *Exp. Physiol.* 87, 633–642. doi: 10.1113/eph8702437
- Regan, R. E., Fisher, J. A., and Duffin, J. (2014). Factors affecting the determination of cerebrovascular reactivity. *Brain Behav.* 4, 775–788. doi: 10.1002/brb3.275
- Rupp, T., Esteve, F., Bouzat, P., Lundby, C., Perrey, S., Levy, P., et al. (2014). Cerebral hemodynamic and ventilatory responses to hypoxia, hypercapnia, and hypocapnia during 5 days at 4,350 m. *J. Cereb. Blood Flow Metab.* 34, 52–60. doi: 10.1038/jcbfm.2013.167
- Schoene, R. B., Roach, R. C., Hackett, P. H., Sutton, J. R., Cymerman, A., and Houston, C. S. (1990). Operation Everest II: ventilatory adaptation during gradual decompression to extreme altitude. *Med. Sci. Sports Exerc.* 22, 804–810. doi: 10.1249/00005768-199012000-00012
- Serrador, J. M., Picot, P. A., Rutt, B. K., Shoemaker, J. K., and Bondar, R. L. (2000). MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* 31, 1672–1678. doi: 10.1161/01.STR.31.7.1672
- Severinghaus, J. W. (1965). Role of cerebrospinal fluid pH in normalization of cerebral blood flow in chronic hypocapnia. *Acta Neurol. Scand. Suppl.* 14, 116–120. doi: 10.1111/j.1600-0404.1965.tb01967.x
- Severinghaus, J. W., Chiodi, H., Eger, E. I. N., Brandstater, B., and Hornbein, T. F. (1966). Cerebral blood flow in man at high altitude: role of cerebrospinal fluid pH in normalization of flow in chronic hypocapnia. *Circ. Res.* 19, 274–282. doi: 10.1161/01.RES.19.2.274
- Severinghaus, J. W., and Lassen, N. (1967). Step hypocapnia to separate arterial from tissue PCO₂ in the regulation of cerebral blood flow. *Circ. Res.* 20, 272–278. doi: 10.1161/01.RES.20.2.272
- Siesjö, B. K. (1972). The regulation of cerebrospinal pH. *Kidney Int.* 1, 360–374. doi: 10.1038/ki.1972.47
- Skinhoj, E. (1966). Regulation of cerebral blood flow as a single function of the interstitial pH in the brain. A hypothesis. *Acta Neurol. Scand.* 42, 604–607. doi: 10.1111/j.1600-0404.1966.tb01211.x
- Smith, C. A., Jameson, L. C., and Dempsey, J. A. (1988). Effects of altered CSF [H⁺] on ventilatory responses to exercise in the awake goat. *J. Appl. Physiol.* (1985) 65, 921–927.
- Somogyi, R. B., Preiss, D., Vesely, A., Fisher, J. A., and Duffin, J. (2005). Changes in respiratory control after 5 days at altitude. *Respir. Physiol. Neurobiol.* 145, 41–52. doi: 10.1016/j.resp.2004.11.004
- Subudhi, A. W., Bourdillon, N., Bucher, J., Davis, C., Elliott, J. E., Eutermoster, M., et al. (2014a). AltitudeOmics: the integrative physiology of human acclimatization to hypobaric hypoxia and its retention upon reascent. *PLoS ONE* 9:e92191. doi: 10.1371/journal.pone.0092191
- Subudhi, A. W., Fan, J. L., Evero, O., Bourdillon, N., Kayser, B., Julian, C. G., et al. (2014b). AltitudeOmics: cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness. *J. Appl. Physiol.* (1985) 116, 724–729. doi: 10.1152/japplphysiol.00880.2013
- Subudhi, A. W., Fan, J. L., Evero, O., Bourdillon, N., Kayser, B., Julian, C. G., et al. (2014c). AltitudeOmics: effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery. *Exp. Physiol.* 99, 772–781. doi: 10.1113/expphysiol.2013.075184
- Sullivan, G. M., and Feinn, R. (2012). Using effect size-or why the P value is not enough. *J. Grad. Med. Educ.* 4, 279–282. doi: 10.4300/JGME-D-12-00156.1
- Toda, N., Hatano, Y., and Mori, K. (1989). Mechanisms underlying response to hypercapnic and bicarbonate of isolated dog cerebral arteries. *Am. J. Physiol.* 257, H141–H146.
- Valdeuza, J. M., Balzer, J. O., Villringer, A., Vogl, T. J., Kutter, R., and Einhüpl, K. M. (1997). Changes in blood flow velocity and diameter of the middle cerebral artery during hyperventilation: assessment with MR and transcranial Doppler sonography. *AJNR Am. J. Neuroradiol.* 18, 1929–1934.
- Verbree, J., Bronzwaer, A. S., Ghariq, E., Versluis, M. J., Daemen, M. J., van Buchem, M. A., et al. (2014). Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra high-field MRI. *J. Appl. Physiol.* (1985) 117, 1084–1089. doi: 10.1152/japplphysiol.00651.2014
- Villien, M., Bouzat, P., Rupp, T., Robach, P., Lamalle, L., Tropres, I., et al. (2013). Changes in cerebral blood flow and vasoreactivity to CO₂ measured by arterial spin labeling after 6 days at 4350m. *Neuroimage* 72, 272–279. doi: 10.1016/j.neuroimage.2013.01.066
- Warner, D. S., Turner, D. M., and Kassell, N. F. (1987). Time-dependent effects of prolonged hypercapnia on cerebrovascular parameters in dogs: acid-base chemistry. *Stroke* 18, 142–149. doi: 10.1161/01.STR.18.1.142
- Willie, C. K., MacLeod, D. B., Smith, K. J., Lewis, N. C., Foster, G. E., Ikeda, K., et al. (2015). The contribution of arterial blood gases in cerebral blood flow regulation and fuel utilization in man at high altitude. *J. Cereb. Blood Flow Metab.* 35, 873–881. doi: 10.1038/jcbfm.2015.4
- Xu, K., and Lamanna, J. C. (2006). Chronic hypoxia and the cerebral circulation. *J. Appl. Physiol.* 100, 725–730. doi: 10.1152/japplphysiol.00940.2005

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2016 Fan, Subudhi, Duffin, Lovering, Roach and Kayser. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.