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Regulation of cerebral blood flow by arterial PCO_2 independent of metabolic acidosis at 5,050 m

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Summary:

- We investigated the influence of arterial PCO_2 ($PaCO_2$) with and without experimentally altered pH on cerebral blood flow (CBF) regulation at sea level and with acclimatization to 5,050 m.
- At sea level and high-altitude, we assessed stepwise alterations in $PaCO_2$ following metabolic acidosis (via two days of oral acetazolamide; ACZ) with and without acute restoration of pH (via intravenous sodium bicarbonate; ACZ+HCO_3^-).
- Total resting CBF was unchanged between trials within each altitude even though arterial pH and [HCO_3^-] (i.e., buffering capacity) were effectively altered.
- The cerebrovascular responses to changes in arterial [H^+]/pH were consistent with the altered relationship between $PaCO_2$ and [H^+]/pH following ACZ at high-altitude (i.e., leftward x-intercept shifts).
- Absolute cerebral blood velocity (CBV) and the sensitivity of CBV to $PaCO_2$ was unchanged between trials at high-altitude, indicating that CBF is acutely regulated by $PaCO_2$ rather than *arterial* pH.

Key Words: Cerebral blood flow, acid-base balance, high-altitude, CO_2 reactivity, acetazolamide, sodium bicarbonate, metabolic acidosis

ABSTRACT

Alterations in acid-base balance with progressive acclimatization to high-altitude have been well-established; however, how respiratory alkalosis and resultant metabolic compensation interact to regulate cerebral blood flow (CBF) is uncertain. We addressed this via three separate experimental trials at sea level and following partial acclimatization (14 to 20 days) at 5,050 m; involving: 1) resting acid-base balance (control); 2) following metabolic acidosis via two days of oral acetazolamide at 250 mg every 8 hours (ACZ; pH: Δ -0.07±0.04 and base excess: Δ -5.7±1.9 $mEq \cdot l^{-1}$, trial effects: $P<0.001$ and $P<0.001$, respectively); and 3) after acute normalization of arterial acidosis via intravenous sodium bicarbonate (ACZ+HCO_3^-; pH: Δ -0.01±0.04 and base excess: Δ -1.5±2.1 $mEq \cdot l^{-1}$, trial effects: $P=1.000$ and $P=0.052$, respectively). Within each trial, we utilized transcranial Doppler ultrasound to assess the cerebral blood velocity (CBV) response to stepwise alterations in arterial PCO_2 ($PaCO_2$); i.e., cerebrovascular CO_2 reactivity. Resting CBF (via Duplex ultrasound) was unaltered between trials within each altitude, indicating that respiratory compensation (i.e., Δ -3.4±2.3 mmHg $PaCO_2$, trial effect: $P<0.001$) was sufficient to offset any elevations in CBF induced via the ACZ-mediated metabolic acidosis. Between trials at high-altitude, we observed consistent *leftward* shifts in both the $PaCO_2$-pH and CBV-pH responses across the CO_2 reactivity tests with experimentally *reduced* arterial pH via ACZ. When indexed against $PaCO_2$ – rather than pH – the absolute CBV and sensitivity of CBV-$PaCO_2$ was unchanged between trials at high-altitude. Taken together, following acclimatization, CO_2-mediated changes in cerebrovascular tone rather than *arterial* $[H^+]$/pH is integral to CBF regulation at high-altitude.

Word Count: 250/250

INTRODUCTION

The cerebral vasculature is exceptionally sensitive to alterations in the partial pressure of arterial carbon dioxide ($PaCO_2$) (Hoiland *et al.*, 2019) such that increases and decreases in $PaCO_2$ (i.e., hyper- and hypocapnia) rapidly increase and decrease cerebral blood flow (CBF), respectively (Kety & Schmidt, 1948). The integrative relationship between $PaCO_2$ and pH on CBF regulation acts to stabilize CO_2 gradients and thus regulate pH across the blood-brain-barrier; that is, alterations in $PaCO_2$ provoke inverse changes in pH (Fencl *et al.*, 1969; reviewed in: Hoiland *et al.*, 2019). Although acute changes in respiratory acidosis/alkalosis elicit changes in interstitial/intracellular pH (Fencl *et al.*, 1966; Betz & Heuser, 1967; Arieff *et al.*, 1976), the CSF pH is stable across *chronic* metabolic acidosis and alkalosis to support a narrow range of extravascular pH levels irrespective of marked changes in arterial pH (Mitchell *et al.*, 1965; Fencl *et al.*, 1969; reviewed in: Siesjö, 1972). It is noteworthy that $PaCO_2$-mediated cerebrovascular responses are dependent on the rapid diffusion of CO_2 across the vascular wall to alter perivascular extracellular pH rather than direct changes in arterial pH *per se* (Wolff & Lennox, 1930; Lambertsen *et al.*, 1961; Severinghaus & Lassen, 1967; Betz & Heuser, 1967; Wahl *et al.*, 1970; Kontos *et al.*, 1977*b*; 1977*a*). With this view, at least in the context of acute metabolic alkalosis, CBF regulation is dependent on $PaCO_2$ rather than arterial pH *per se* (Caldwell *et al.*, 2021); however, whether this finding is consistent following acclimatization to high-altitude, where metabolic compensation for the prevailing respiratory alkalosis occurs, merits investigation.

Alterations in acid-base balance during high-altitude exposure are well-reported (Dempsey *et al.*, 1974; Forster *et al.*, 1975; Weiskopf *et al.*, 1976). With ascent to high-altitude, the initial hypoxic ventilatory response reduces $PaCO_2$ and arterial $[H^+]$ (i.e., respiratory alkalosis) and, as such, arterial/CSF pH is elevated (Severinghaus *et al.*, 1963; reviewed in: Hoiland *et al.*, 2018). This respiratory alkalosis is partially compensated by renal excretion of bicarbonate (HCO_3^-) that begins in the first 1-2 days across progressive acclimatization; e.g., 1-2 weeks (Gledhill *et al.*, 1975; Dempsey *et al.*, 1978; Krapf *et al.*, 1991). Acetazolamide (ACZ) is a carbonic anhydrase inhibitor that accelerates the acclimatization process (Bärtsch & Swenson, 2013; Swenson, 2014) by increasing ventilation and accelerated renal excretion of HCO_3^- to induce metabolic acidosis (Kronenberg & Cain, 1968; Teppema *et al.*, 2010). At sea level,

studies indicate that the initial marked 40-50% increase in resting CBF mediated by transient extracellular acidosis provoked by a single oral dose of 1,000 mg ACZ is normalized within two days of chronic ACZ (10 days of 500 mg twice daily) via respiratory compensation; i.e., progressive 30% reduction in alveolar PCO_2 (Lassen *et al.*, 1987; Friberg *et al.*, 1990). These results emphasize that the influence of ACZ on CBF regulation is likely dependent on the countervailing balance between: 1) metabolic acidosis-induced cerebral *vasodilation* (Fencl *et al.*, 1969; Kontos *et al.*, 1977*b*); and 2) hyperventilation-induced hypocapnic cerebral *vasoconstriction* (Willie *et al.*, 2012; 2015).

According to the Henderson-Hasselbalch equation: $pH = 6.1 + \log [HCO_3^-] / (0.0314 \times PCO_2)$ (Hasselbalch, 1916), reductions in arterial $[HCO_3^-]$ at high-altitude would decrease buffering capacity; that is, a given change in $PaCO_2$ will elicit a larger change in arterial $[H^+]$/pH (Siesjö, 1972). As such, appropriate changes in cerebrovascular CO_2 reactivity (i.e., change in CBF for a given change in $PaCO_2$) with respect to changes in buffering capacity are essential to tightly regulate cerebral interstitial pH to support critical enzymatic function (Fencl *et al.*, 1966; reviewed in: Fencl & Rossing, 1989). Likewise, if the cerebral vasculature is acutely regulated by $PaCO_2$ *per se* (Schieve & Wilson, 1953; Lambertsen *et al.*, 1961; Caldwell *et al.*, 2021), then alterations in the buffering capacity of $PaCO_2$ and $[H^+]$/pH at high-altitude will result in consistent changes with CBF and $[H^+]$/pH (i.e., rightward x-intercept shifts). Previous reports indicate either attenuated (Ainslie *et al.*, 2008), unchanged (Ainslie & Burgess, 2008; Rupp *et al.*, 2014; Willie *et al.*, 2015), or augmented (Fan *et al.*, 2010; Lucas *et al.*, 2011; Flück *et al.*, 2015) cerebrovascular CO_2 reactivity with initial ascent and partial acclimatization to high-altitude. These disparate findings are likely attributable to differences in approaches to index cerebrovascular CO_2 reactivity, severity of altitude, ascent profile, stage of acclimatization, and the prevailing compensatory changes in the buffering capacity of $PaCO_2$ and $[H^+]$/pH at high-altitude (Crawford & Severinghaus, 1978; Mathew *et al.*, 1983; Fan *et al.*, 2015).

This study investigated the interaction between acid-base balance on resting CBF and cerebrovascular CO_2 reactivity via three separate experimental trials at sea level and following partial acclimatization (14 to 20 days) at 5,050 m, involving: 1) resting acid-base balance (control); 2) following two days of oral acetazolamide dosing (ACZ; 250 mg per 8 hours); and 3) after acute normalization of arterial pH ($ACZ+HCO_3^-$). To account for the relative metabolic acidosis elicited by ACZ, we utilized an intravenous infusion of sodium bicarbonate ($NaHCO_3^-$)

to normalize arterial pH with a partial restoration of $PaCO_2$. At least at sea level, intravenous $NaHCO_3^-$ infusion provokes progressive increases in $PaCO_2$ via respiratory suppression (Gesell *et al.*, 1930; Shock & Hastings, 1935; Bernthal, 1937; Hesser, 1949; Singer *et al.*, 1956) to partially compensate for the associated metabolic alkalosis; however, this elevated *arterial* pH is only reflected in the CSF/extracellular pH after several hours (Robin *et al.*, 1958; Bradley & Semple, 1962; Hornbein & Pavlin, 1975; Nattie & Romer, 1978; Abeysekara *et al.*, 2012). Whether these compensatory changes in respiration occur at high-altitude and their resultant influence on cerebrovascular regulation has not been reported. We hypothesized that CBF regulation at rest would correspond to changes in $PaCO_2$ (e.g., respiratory compensation) rather than arterial pH (e.g., pharmacologically induced) between trials at sea level and high-altitude. Additionally, we hypothesized that the cerebrovascular responses to changes in $[H^+]$/pH would be consistent with the altered relationship between $PaCO_2$ and $[H^+]$/pH between altitudes and within the acute acid-base trials.

METHODS

Ethical Approval

The study was approved by the Clinical Ethical Review Board at the University of British Columbia (H11-03287) and the Nepal Health Medical Research Council. All experimental procedures were conducted in accordance with the Declaration of Helsinki (except registration in a database). Following verbal and written explanation of the study, written informed consent was provided by all volunteers.

Participants

Eleven healthy adults (n = 9 males/ 2 females; 28 ± 6 years, 175 ± 6 cm, 77 ± 14 kg, 25 ± 4 kg/m^2) participated in this study at sea level (SL). Ten healthy adults (n = 7 males/ 3 females; 29 ± 5 years, 175 ± 6 cm, 74 ± 12 kg, 24 ± 4 kg/m^2) participated in this study at high-altitude (HA). Participants had no history of cardiovascular, cerebrovascular, or respiratory disease and were not taking any cardiovascular medications.

Experimental Overview

This study was part of a larger research expedition conducted in April-June 2012. As such, participants were involved in a number of studies conducted during pre-testing in Kelowna and during the 3 weeks at the Ev-K2-CNR Pyramid Laboratory. The recovery time between the various testing sessions was managed to avoid any cross-over effects between multiple experiments (e.g., >48 hours between all drug and/or exercise intervention studies). The experimental questions addressed in this study were *a priori* driven; however, a subset of participants' resting arterial blood gas variables have been reported elsewhere in a separate report (at sea level only) on the influence of ACZ on the pulmonary vascular pressure response to acute hypoxia and blood flow through intrapulmonary arteriovenous anastomoses (Tremblay *et al.*, 2015).

Ascent to High-Altitude

All variables and measurements were obtained at the University of British Columbia Okanagan Campus in Kelowna, BC, Canada (SL: 344 m, barometric pressure (Pb) 732 ± 16 mmHg) and following 14 to 20 days at the Ev-K2-CNR Pyramid Laboratory, Khumbu Valley, Nepal (HA: 5,050 m, Pb = 413 ± 4 mmHg). Participants spent 7 days in Kathmandu (1,338 m) acclimatizing before flying to Lukla (2,860 m) to begin the trek to 5,050 m over 6-8 days (rest days: Namche Bazaar, 3,440 m; Pengboche, 3,995 m; Pheriche, 4,371 m). Additionally, during the first 6-7 days of ascent to 5,050 m, participants were given low-dose acetazolamide (125 mg, oral) twice a day as an acute mountain sickness prophylactic (Basnyat *et al.*, 2006; Ritchie *et al.*, 2012). Treatment of acetazolamide was discontinued on day 8 of the trek at 4,371 m to allow sufficient time (e.g., >24 hours) for the drug to clear participants' system before the control trial at 5,050 m (Ritschel *et al.*, 1998; Richalet *et al.*, 2005). This approach was utilized to provide a safe ascent of the experimental volunteers at 5,050 m.

Protocol 1

At SL and following 14 to 20 days at HA participants first completed a control visit including a standardized intra-cranial cerebrovascular CO_2 reactivity (CVR) test including stepwise iso-oxic alterations in $PaCO_2$ (hypo- and hypercapnia) in the following order: -10, -5, +0, +5, +10, +15 mmHg $PaCO_2$ via dynamic end-tidal forcing. The alterations in $PaCO_2$ were calculated from the resting eupneic breathing end-tidal values obtained prior to each of the three experimental trials

at each altitude. Participants were unable to tolerate +15 mmHg $PaCO_2$ at 5,050 m; therefore, the hypercapnic CVR range was completed up to +10 mmHg $PaCO_2$ at HA. Each stage of the cerebrovascular CO_2 reactivity protocol lasted approximately 3 minutes to allow a steady-state responsiveness to be achieved (Carr *et al.*, unpublished). All cardiorespiratory, cerebrovascular, and arterial blood gas variables presented were measured within the last minute of each stage, representative of a steady-state.

Protocol 2 & 3

Following the control visit, participants were prescribed an oral dose of ACZ (250 mg) every 8 hours for 2 days before their next visit. The last dose of ACZ was taken 1 hour before experimentation. The cerebrovascular CO_2 reactivity protocol was then repeated twice (separated by at least 30 minutes) without intravenous $NaHCO_3^-$ (ACZ) and with intravenous $NaHCO_3^-$ (ACZ+HCO_3^-). To allow for experimental alteration of arterial pH from a setting of relative metabolic acidosis caused by ACZ, the 8.4% intravenous $NaHCO_3^-$ solution (Hospira, Montreal, Quebec, Canada) was delivered over a 15-minute infusion to acutely restore arterial pH to resting levels.

Arterial Blood Sampling

At SL, arterial blood samples (approx. 1.0 mL) were collected from the radial artery under local anesthesia (Lidocaine, 1.0%) using a 23-gauge needle and self-filling pre-heparinized syringe (SafePICO syringes, Radiometer, Copenhagen, Denmark). At HA, a radial artery catheter (20-gauge; Arrow, Markham, ON, Canada) was placed under local anesthesia (Lidocaine, 1.0%) and ultrasound guidance. The radial artery catheter was attached to an in-line waste-less blood sampling system (Edwards Lifesciences, TruWave VAMP, CA, USA) for repeated measurements. All blood gas samples were analyzed immediately using a calibrated blood gas analyzer (ABL90 FLEX, Radiometer). This analysis included measurements of the partial pressures of arterial carbon dioxide ($PaCO_2$) and oxygen (PaO_2), arterial oxygen saturation (SaO_2), bicarbonate ion concentration ([HCO_3^-]), hydrogen ion concentration ([H^+]), hemoglobin concentration ([Hb]), hematocrit (HCT) and arterial pH. All samples were heated/corrected to an assumed resting body temperature of 37.0°C.

Blood gas analyzers do not typically have the capacity to directly measure ($[HCO_3^-]$); instead, it is calculated from measured $PaCO_2$ and pH, using the Henderson-Hasselbalch equation (Hasselbalch, 1916). The pKa (i.e., -log of the acid dissociation constant) at 37.0°C of 6.1 (Cullen *et al.*, 1925) and the solubility factor for dissolved CO_2 plus carbonic acid (H_2CO_3) at 37.0°C in plasma of 0.0314 $mmol \cdot l^{-1}$ per mmHg $PaCO_2$ were used:

$$pH = 6.1 + \log [HCO_3^-] / (0.0314 \times PaCO_2)$$

At SL, arterial blood samples were obtained from the radial artery prior to each cerebrovascular CO_2 reactivity trial (e.g., control, ACZ, ACZ+HCO_3^-) in a subgroup of participants (n=7) and again in five participants following the ACZ+HCO_3^- protocol to confirm that arterial pH and $[HCO_3^-]$ were maintained for the duration of the experimental protocol. At HA, arterial blood samples were obtained in all 10 participants at rest prior to and during the cerebrovascular CO_2 reactivity protocols for the control and ACZ trials; the ACZ+HCO_3^- trial was conducted on the next day at HA and arterial blood samples were obtained in a subgroup of participants (n=5).

At both SL and HA the deficit in $[HCO_3^-]$ was calculated from resting arterial $[HCO_3^-]$ taken with and without ACZ and using body mass to calculate the required dosage of $NaHCO_3^-$ with the below equations (Kollef & Isakow, 2012).

Apparent volume of distribution = total body weight (kg) × (0.4 + (2.4 / ACZ $[HCO_3^-]$))
Target change in $[HCO_3^-]$ = resting $[HCO_3^-]$ – ACZ $[HCO_3^-]$
mEq of $NaHCO_3^-$ = Apparent volume of distribution × target change in $[HCO_3^-]$ × 0.5

Arterial blood samples were obtained following $NaHCO_3^-$ infusion to confirm sufficient normalization to control values. In the event that $[HCO_3^-]$ was not completely restored to resting levels additional $NaHCO_3^-$ was administered and arterial $[HCO_3^-]$ levels were reassessed before experimentation to confirm adequate restoration. The order of experiments was not randomized because of the lasting effects of ACZ and $NaHCO_3^-$.

Cardiorespiratory

Breath-by-breath CO_2 and O_2 were sampled at the mouth and recorded using a gas analyzer calibrated prior to each experimental session (ML206, ADInstruments, CO, USA). The partial pressures of end-tidal CO_2 and O_2 (i.e., $P_{ET}CO_2$ and $P_{ET}O_2$, respectively) were calculated in LabChart (ADInstruments) using peak detection analysis with correction for daily barometric pressure at BTPS. Both $P_{ET}CO_2$ and $P_{ET}O_2$ were controlled using a custom-designed dynamic end-tidal forcing system to effectively regulate end-tidal gases across wide ranges of $P_{ET}CO_2$ and $P_{ET}O_2$ independent of ventilation ($\dot{V}_E$); this device has previously been described in detail elsewhere (Tymko *et al.*, 2015; 2016). Notably, this method of arterial blood gas alteration attenuates the end-tidal-to-arterial PCO_2 gradient, precludes any influence of $\dot{V}_E$ on cerebrovascular CO_2 reactivity (Howe *et al.*, 2020), and therefore provides an accurate stimulus-response relationship (Fisher, 2016; Fisher *et al.*, 2018). Respiratory flow, tidal volume (V_T), and respiratory frequency (f_R) were measured by a pneumotachograph (HR 800 L, Hans Rudolph, Shawnee, KS, USA). Instantaneous minute ventilation ($\dot{V}_E$ in liters per minute) was determined as the product of breath-by-breath inspired volume (V_T; calculated from the integral of the flow signal) and respiratory frequency (f_R, in breaths per minutes; calculated by 60/period of the flow signal).

Cardiovascular

At HA, beat-by-beat arterial blood pressure was acquired via the radial artery pressure transducer positioned at the height of the right atrium (Edwards Lifesciences, TruWave VAMP, CA, USA). At SL, continuous non-invasive blood pressure was acquired using finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands) and was calibrated prior to data collection using the return-to-flow function and normalized to manual brachial artery blood pressure measurements. The arterial and finger photoplethysmography blood pressure waveforms were averaged to calculate MAP at each altitude, respectively. Heart rate was continuously measured using a lead-II electrocardiogram (ECG; ML132 BioAmp, ADInstruments, CO, USA). Peripheral oxygen saturation (SpO_2) was measured continuously by pulse oximetry (ML320/F; ADInstruments, CO, USA).

Cerebrovascular

At rest, extra-cranial blood velocity and vessel diameter of the left internal carotid artery (ICA) and right vertebral artery (VA) were measured using a 10-MHz multifrequency linear array Duplex ultrasound (Terason t3000; Teratech, Burlington, MA, USA). Pulse-wave mode was used to measure peak blood velocity and arterial diameter was instantaneously measured using B-mode imaging. The ICA blood velocity and vessel diameter were measured ≥1.5 cm from the carotid bifurcation to avoid any turbulent or retrograde flow patterns, while VA blood velocity and diameter were measured between C4-C5 or C5-C6. The vessel location was decided on an individual basis to allow for reliable image acquisition, with the same location and consistent insonation angle (60°) repeated within participants and between trials. Our between-day coefficients of variation for Q_{ICA} and Q_{VA} are 5% and 11%, respectively (Willie *et al.*, 2012). Intra-cranial cerebral blood velocity (CBV) was assessed at rest and during CO_2 reactivity via transcranial Doppler (TCD) ultrasound (Spencer Technologies, Seattle, WA, USA), as an index of CBF, in the left middle cerebral artery (MCA) and right posterior cerebral artery (PCA). The 2-MHz TCD probes were attached to a specialized headband (model M600 bilateral head frame, Spencer Technologies), and each vessel was insonated through the trans-temporal window, using previously described location and standardization techniques (Willie *et al.*, 2011). Our between-day coefficients of variation for MCA*v* and PCA*v* are 3% and 2%, respectively (Smith *et al.*, 2012).

Data Analyses

Cardiovascular and respiratory measures were sampled continuously at 1000 Hz using an analogue-to-digital converter (Powerlab/16SP ML795; ADInstruments, Colorado Springs, CO, USA) and data were interfaced with LabChart (Version 7.1) and analyzed offline. Cardiovascular and respiratory variables presented are 1-minute averages during steady-state conditions after ≥2 minutes at each stage of the CO_2 reactivity protocol. The Q_{ICA} and Q_{VA} recordings were at least 1-minute for each measurement (Thomas *et al.*, 2015). Duplex ultrasound recordings were screen captured and saved for offline analysis using custom edge-detection and wall tracking software (BloodFlow Analysis, version 5.1). This analysis method utilizes integration of diameter and velocity traces to calculate mean beat-to-beat flow at 30 Hz independent of observer bias (Woodman *et al.*, 2001).

Blood flow was calculated as:

Q (mL·min^{-1}) = peak envelope blood velocity/ 2 × (π(0.5 × diameter)^2) × 60.

Global cerebral blood flow (gCBF) was calculated as:

gCBF (mL·min^{-1}) = 2 × (Q_{ICA} + Q_{VA})

Arterial oxygen content (CaO_2) was calculated as:

CaO_2 (mL·dL^{-1}) = [Hb] × 1.34 × [SaO_2 (%)/ 100] + 0.003 × PaO_2

Where 1.34 is the O_2 binding capacity of hemoglobin and 0.003 is the solubility of O_2 dissolved in blood (Lumb, 2016; West & Luks, 2020).

Cerebral oxygen delivery (CDO_2) was calculated as:

CDO_2 (mL·min^{-1}) = gCBF × CaO_2

MCA or PCA DO_2 (au) = MCAv or PCAv × CaO_2

Cerebrovascular conductance (CVC) was calculated as:

CVC (mL·min^{-1}·mmHg^{-1}) = gCBF, Q_{ICA}, Q_{VA}, MCAv, or PCAv / MAP

Statistical Analyses

All data are presented as mean ± SD. Statistical analyses were performed using SPSS software (IBM statistics, Version 23.0) and statistical significance was set at $P \leq 0.05$. Comparisons were made between SL and HA at rest between trials (control, ACZ, ACZ+HCO_3^-), and between $PaCO_2$ stages within elevation. A linear mixed-model analysis with compound symmetry covariance structure with fixed effects of trial (control, ACZ, ACZ+HCO_3^-) and altitude (SL vs. HA) was used to compare arterial blood gas, cardiorespiratory, and cerebrovascular variables at rest. Resting MAP, $PaCO_2$, pH, [HCO_3^-], and CaO_2 were added as covariates alongside trial and altitude as fixed effects and subjects as a random effect for resting gCBF. The selected variables were chosen as they are considered important regulators of CBF in humans (Willie *et al.*, 2014) and they each improved the model fit (-2 Log Likelihood), indicating their acceptability in the model. A Bonferroni correction was applied for multiple comparisons when significant

interactions were detected. A linear mixed-model analysis with fixed effects of trial (control, ACZ, ACZ+HCO_3^-) and stage ($PaCO_2$ level) was used during the cerebrovascular CO_2 reactivity protocol for separate SL and HA comparisons. Separate hypo- and hypercapnic CVR was analyzed using linear regression to calculate the individual slope response between cerebrovascular parameters and $P_{ET}CO_2$. A one-tailed, paired Student's t-test was used to compare the individual x-intercept values for the absolute $PaCO_2$ versus pH and MCA*v* versus pH cerebrovascular CO_2 reactivity slopes between each experimental trial at HA.

RESULTS

Arterial blood gases

Between altitudes within trials at rest: As expected, HA resulted in arterial hypoxemia (PaO_2: Δ -53 ± 6 mmHg and SaO_2: Δ -13.4 ± 1.3 %, altitude effects: $P < 0.001$ and $P < 0.001$, respectively; **Table 1**) and respiratory alkalosis ($PaCO_2$: Δ -14.7 ± 2.3 mmHg and pH: Δ +0.03 ± 0.04, altitude effects: $P < 0.001$ and $P = 0.002$, respectively; **Table 1**) with partial metabolic compensation ([HCO_3^-]: Δ -7.6 ± 1.4 mEq·l^{-1}, within trials all $P < 0.001$; **Table 1**). Overall, CaO_2 was lower at HA versus SL during control and ACZ trials ($P < 0.001$ and $P < 0.001$, respectively; **Table 1**) with no change between altitudes during the ACZ+HCO_3^- trial ($P = 0.433$; **Table 1**).

Between trials across altitudes at rest: Across altitudes, arterial pH was lower following ACZ versus control (Δ -0.07 ± 0.04, trial effect: $P < 0.001$; **Table 1**) and ACZ+HCO_3^- (Δ -0.06 ± 0.04, trial effect: $P < 0.001$; **Table 1**); that is, ACZ+HCO_3^- effectively restored arterial pH to control values at both SL and HA. Following ACZ, arterial [HCO_3^-] was lower at SL (Δ -6.3 ± 2.0 mEq·l^{-1}, $P < 0.001$; **Table 1**) and HA (Δ -3.9 ± 2.1 mEq·l^{-1}, $P < 0.001$; **Table 1**); as such, $NaHCO_3^-$ infusion effectively normalized arterial [HCO_3^-] to control values at each altitude ($P = 0.279$ and $P = 0.060$, respectively; **Table 1**). Following ACZ at SL and HA, $PaCO_2$ was lower (Δ -3.4 ± 2.3 mmHg, trial effect: $P < 0.001$; **Table 1**) and PaO_2 was higher (Δ +6.3 ± 6.5 mmHg, trial effect: $P = 0.003$; **Table 1**) with no change between control and ACZ+HCO_3^- trials (trial effect: $P = 0.053$ and $P = 0.458$, respectively; **Table 1**). At SL, CaO_2 was higher following ACZ versus control (Δ +1.2 ± 1.0 mL·dL^{-1}, $P < 0.001$; **Table 1**) and ACZ+HCO_3^- (Δ +1.2 ± 1.0

377 $mL \cdot dL^{-1}$, P = 0.001; **Table 1**) trials. At HA, CaO_2 was also higher following ACZ (Δ +1.8 ± 1.1
378 $mL \cdot dL^{-1}$, P < 0.001; **Table 1**) and ACZ+HCO_3^- (Δ +1.7 ± 1.4 $mL \cdot dL^{-1}$, P < 0.001; **Table 1**)
379 versus the control trial.
380
381 *Cardiorespiratory*
382 There was no interaction between the resting $\dot{V}_E$, HR, and MAP responses between control,
383 ACZ, and ACZ+HCO_3^- trials and SL versus HA (all P > 0.05; **Table 2**). Throughout the CO_2
384 reactivity tests, there was no interaction between the $\dot{V}_E$, HR, and MAP responses between trials
385 at either altitude (all P > 0.05). Across altitudes, MAP was lower at rest and throughout the CO_2
386 reactivity tests during the ACZ trial compared to control and ACZ+HCO_3^- trials (trial effects: all
387 P < 0.05; **Table 2**).
388
389 *Cerebrovascular*
390 There was no interaction between the resting CDO_2, *g*CBF, and *g*CBF_{CVC} responses between
391 control, ACZ, and ACZ+HCO_3^- trials and SL versus HA (all P > 0.05; **Table 2**). Resting CDO_2,
392 *g*CBF, and *g*CBF_{CVC} were all higher at HA versus SL (altitude effects: all P < 0.05; **Table 2**).
393 Further, covariate analysis revealed no significant influence of resting $PaCO_2$, pH, or [HCO_3^-] on
394 resting CDO_2, *g*CBF, and *g*CBF_{CVC} responses between trials and altitudes (all P > 0.05). As
395 such, there was no correlation between the absolute resting $PaCO_2$, [H^+], or pH and the
396 respective *g*CBF within trials at each altitude (all P > 0.05).
397
398 *Regulation of resting cerebral blood flow by arterial PCO_2, pH, and H^+:* **Figure 1** provides
399 context for the intra-individual variability in resting acid-base balance (i.e., metabolic/respiratory
400 compensation) and respective CBF regulation within the ACZ and ACZ+HCO_3^- experimental
401 trials. As ACZ provokes reductions in both arterial pH and $PaCO_2$ (**Table 1**) – via metabolic
402 acidosis and elevated respiration – it is important to consider the directional alterations in *g*CBF
403 responses with these respective competing changes in $PaCO_2$ and [H^+]/pH between trials at SL
404 and HA. Overall, an unchanged *g*CBF response corresponded with a higher arterial [H^+] (i.e.,
405 lower pH) as well as a reduction in $PaCO_2$ following ACZ; that is, respiratory compensation (i.e.,
406 Δ $PaCO_2$), rather than the prevailing arterial [H^+]/pH, is sufficient to offset any changes in *g*CBF
407 with ACZ and ACZ+HCO_3^- acid-base alterations at SL and HA.

High-altitude MCAv CO_2 reactivity regulation: Throughout the CO_2 reactivity tests at HA, arterial pH was lower during the ACZ trial than control (Δ -0.08 ± 0.01, trial effect: $P < 0.001$; **Figure 2**) and ACZ+HCO_3^- (Δ -0.03 ± 0.02, trial effect: $P < 0.001$; **Figure 2**). The arterial pH was lower during the ACZ+HCO_3^- versus control trial CO_2 reactivity test (Δ -0.05 ± 0.02, trial effect: $P < 0.001$; **Figure 2**) even though arterial pH was not different at rest between ACZ+HCO_3^- and control trials ($P = 0.060$). Across $PaCO_2$ stages, CaO_2 was higher than the control trial with both ACZ (Δ +1.4 ± 0.4 mL·dL^{-1}, trial effect: $P < 0.001$) and ACZ+HCO_3^- (Δ +1.7 ± 0.5 mL·dL^{-1}, trial effect: $P < 0.001$); as such, MCA DO_2 was not different between trials (trial effect: $P = 0.622$). Across the full range of $PaCO_2$ alterations, there was no difference between the sensitivity (i.e., slope) of the absolute MCA*v* response to changes in $PaCO_2$, [H^+], or pH ($P = 0.156$, $P = 0.238$, and $P = 0.073$, respectively) across trials at HA (**Figure 2 A & B**). Additionally, absolute MCA*v* was not different at each stage of $PaCO_2$ between trials at HA ($P = 0.913$).

Hypocapnic versus hypercapnic reactivity: Within altitudes at SL and HA, there was no difference between either the absolute MCA*v* or PCA*v* (each covariate adjusted by MAP; all $P < 0.001$) versus $P_{ET}CO_2$/$PaCO_2$ responses, respectively across the full range of CO_2 reactivity (i.e., inclusive of hypo- and hypercapnia) between control, ACZ, and ACZ+HCO_3^- (all $P > 0.05$; **Figure 3A & 3B**). Across trials, the separate hypocapnic and hypercapnic relative CVR slopes were higher at HA versus SL for MCA*v*, PCA*v*, MCA_{CVC}, and PCA_{CVC} (altitude effects: all $P < 0.05$; **Table 3 & Figure 3C**). Across altitudes, absolute and relative MCA*v* hypercapnic CVR were both higher during the control trial than ACZ and ACZ+HCO_3^- with no influence of altitude *per se* (trial effects: $P = 0.004$ and $P = 0.005$; **Tables 3-4 & Figure 3D**). At HA, relative PCA_{CVC} hypocapnic CVR was lower during the ACZ+HCO_3^- trial than control (-15 ± 24%, $P = 0.028$) and ACZ (-21 ± 18%, $P = 0.003$). At SL, the absolute hypocapnic CVR was higher during the ACZ+HCO_3^- trial than control for MCA*v* (+0.8 ± 0.7 cm·s^{-1} per mmHg, $P = 0.003$), MCA_{CVC} (+0.01 ± 0.01 cm·s^{-1} per mmHg, $P = 0.004$), and PCA_{CVC} (+0.01 ± 0.00 cm·s^{-1} per mmHg, $P = 0.021$) (**Table 4**). These between trial differences in the absolute hypocapnic CVR responses to ACZ+HCO_3^- were not apparent at HA.

DISCUSSION

The results of this study indicate that in the context of acute and chronic changes in arterial pH the CBF response is consistent with changes in $PaCO_2$ rather than the prevailing arterial $[H^+]$/pH *per se*. These data support the view that the buffering capacity of extracellular rather than intravascular arterial $[H^+]$/pH gradients regulate CBF as cerebrovascular CO_2 reactivity was consistently higher following partial acclimatization to high-altitude versus sea level irrespective of experimentally controlled metabolic acidosis/alkalosis. This finding is supported by: 1) resting CBF and cerebrovascular CO_2 reactivity were unchanged between trials within each altitude even though arterial pH and $[HCO_3^-]$ (i.e., buffering capacity) were effectively altered; and 2) intra-individual responses at rest indicate reductions in $PaCO_2$ (via respiratory compensation) are sufficient to regulate *g*CBF with metabolic acidosis rather than the countervailing changes in arterial $[H^+]$/pH. Taken together, within the experimental constraints of this study, these findings indicate that CO_2-mediated changes in cerebrovascular regulation rather than *arterial* $[H^+]$/pH is integral in the regulation of CBF in humans following acclimatization to high-altitude.

Cerebrovascular regulation following acute and chronic alterations in acid base balance: Resting CBF was unaltered between trials within each altitude even though arterial pH and $[HCO_3^-]$ (i.e., buffering capacity) were effectively reduced and restored with ACZ and ACZ+HCO_3^- trials, respectively (**Table 1**). These results are inconsistent with previous reports at HA of approximately 25% *increases* in resting CBF following 2 hours of a large oral ACZ ingestion (1.5 g; 3,475 m) (Jensen *et al.*, 1990) or rapid 60-s intravenous infusion (10 mg/kg; 5,050 m) (Fan *et al.*, 2012). It is noteworthy, however, that these studies were performed acutely with a higher relative ACZ dose than the current experiment (2 days; 250 mg per 8 hours) and such doses/intravenous approaches are not ecologically valid at HA (Low *et al.*, 2012; Bärtsch & Swenson, 2013). At SL, acute intravenous ACZ infusion rapidly elevates CBF (Ehrenreich *et al.*, 1961; Hauge *et al.*, 1983; Lassen *et al.*, 1987; Jensen *et al.*, 1990; Fan *et al.*, 2012) without altering cerebral metabolism (Posner & Plum, 1960; Vorstrup *et al.*, 1984); importantly, such acute intravenous protocols do not alter $PaCO_2$ whereas the expected hyperventilatory response was observed in the present study following 2 days oral ACZ (e.g., Δ -5 mmHg $PaCO_2$; **Table 1**). Further, reports indicate ACZ infusion attenuates the regulatory rise in CSF $[HCO_3^-]$ in

response to increases in $PaCO_2$ (Wichser & Kazemi, 1975; Kazemi *et al.*, 1976; Shibata *et al.*, 1976; Kazemi & Choma, 1977); therefore, *intravenous* ACZ may indeed exacerbate reductions in buffering capacity, necessitating an increase in CBF to maintain extravascular pH (Skinhoj, 1966; Severinghaus & Lassen, 1967; Lassen, 1968; Fencl *et al.*, 1969).

Well-established *in vivo* pre-clinical studies show that changes in $PaCO_2$ rather than arterial pH *per se* mediate alterations in CBF by stabilizing perivascular pH (Betz & Heuser, 1967; Wahl *et al.*, 1970; Betz *et al.*, 1973; Kontos *et al.*, 1977*b*; 1977*a*). As such, a rightward shift in the $PaCO_2$-pH relationship at altitude and resultant reduction in buffering capacity (via lower $[HCO_3^-]$) would also result in a rightward shift in the CBF-pH relationship. This view is consistent with recent results from Caldwell and colleagues (2021) that show a rightward x-intercept shift in both the $PaCO_2$-pH and CBF-pH responses following acute metabolic alkalosis via intravenous $NaHCO_3^-$ at SL. Relatedly, between trials at HA, we observed *leftward* shifts in both the $PaCO_2$-pH and CBF-pH relationships with experimentally *reduced* arterial pH (e.g., ACZ; **Figure 2A & 2C**). These data indicate that changes in the x-intercept of CBF versus pH were consistent with the altered relationship between $PaCO_2$ and pH at HA; i.e., the sensitivity of CBF to $PaCO_2$ *per se* was unchanged between trials at HA. At least at SL, previous studies report that metabolic acidosis has an equivalent or additive influence on the hypoxic ventilatory response (Forster & Klausen, 1973; Swenson & Hughes, 1993); however, the absolute change in $PaCO_2$ with ACZ was approximately 60% less at HA than at SL (**Table 1**). As discussed next, the leftward shift in the CBV-$P_{ET}CO_2$ response with ACZ at SL can likely be attributed to the larger absolute change in $PaCO_2$ with ACZ at SL (**Figure 3A**).

Cerebrovascular regulation is not exclusively regulated by arterial pH: These data support recent pre-clinical work which substantiates that CO_2 signalling – via astrocytic CO_2/HCO_3^- transport – mediates CBF regulation (e.g., neurovascular coupling) independently of experimentally altered arterial/extracellular pH (Hosford *et al.*, 2021; *preprint*). Additionally, these results are consistent with evidence that PCO_2-mediated release of ATP (via CO_2-sensitive connexin-26 proteins) independent of extracellular acidosis and Ca^{2+} is integral to the ventilatory response to CO_2 (Huckstepp *et al.*, 2010; Cummins *et al.*, 2020); however, whether this CO_2 signalling is involved with CBF regulation requires investigation. The ACZ-induced reductions in arterial pH would theoretically increase *g*CBF if metabolic acidosis is considered exclusively

(Fencl *et al.*, 1969). Rather, *g*CBF was statistically unaltered between experimental trials within each altitude, indicating that respiratory compensation (i.e., reductions in $PaCO_2$) was sufficient to offset any elevations in *g*CBF expectedly induced by ACZ-mediated metabolic acidosis. In support of this finding, the intra-individual responses indicate that the reductions in $PaCO_2$ are consistent with the directional alterations in *g*CBF with ACZ and ACZ+HCO_3^- trials (**Figure 1**). Following ACZ at SL, resting $PaCO_2$ was reduced by approximately 5 mmHg (**Table 1**); taken together with the well-established hypocapnic CVR of approximately 3-4% per mmHg $PaCO_2$ (Kety & Schmidt, 1948; Ramsay *et al.*, 1993; Ito *et al.*, 2000; 2003; Willie *et al.*, 2012; Coverdale *et al.*, 2014; reviewed in: Hoiland *et al.*, 2019), these data indicate that the extent of respiratory compensation to ACZ is apparently exaggerated with respect to the unaltered *g*CBF. In support of this view, previous reports show a progressive 30% reduction in alveolar PCO_2 following 10 days of oral ACZ (500 mg twice daily); alongside this respiratory compensation, total CBF (via intravenous $xenon^{133}$ technique) was restored to control values within 2 days of ACZ treatment and throughout the following 15 days indicating a countervailing influence of acid-base balance on cerebrovascular regulation (Lassen *et al.*, 1987; Friberg *et al.*, 1990).

The relationship between arterial PCO_2 and H^+/pH predicates cerebrovascular CO_2 reactivity at high-altitude: The present results are consistent with other studies that have reported higher cerebrovascular CO_2 reactivity with initial ascent and partial acclimatization to HA (Fan *et al.*, 2010; 2012; 2014; Flück *et al.*, 2015). Within each altitude, there were selective alterations in absolute and relative hypocapnic CVR during the ACZ+HCO_3^- trial (**Tables 3-4**); such findings are perhaps attributable to relative increases in buffering capacity via exogenously elevated arterial [HCO_3^-] (Siesjö, 1972) and/or direct effects of extracellular [HCO_3^-] on cerebrovascular tone via changes in vascular smooth muscle cell contractility and Ca^{2+} sensitivity (Boedtkjer *et al.*, 2016; Boedtkjer, 2018). As the $NaHCO_3^-$ infusion acutely restored pH – rather than promoting further metabolic alkalosis – it is likely that these effects are due to the exogenous increases in arterial [HCO_3^-] rather than pH *per se*. It is noteworthy that the absolute MCA*v* was not different at each stage of $PaCO_2$ between experimental trials at HA (**Figure 2B**). Pioneering work by Severinghaus and colleagues (1963) revealed tight regulation of CSF pH with chronic hypocapnia and arterial alkalosis following 8 days of acclimatization to 3,800 m – although the capacity of CSF [HCO_3^-] active transport is reportedly controversial, these data illustrate the

importance of interstitial/extracellular pH regulation (Severinghaus *et al.*, 1963; Severinghaus, 1965; Mitchell *et al.*, 1965; Pappenheimer1970, 1970; Hasan & Kazemi, 1976; Kazemi & Choma, 1977; Bledsoe *et al.*, 1981). Overall, the hyperventilatory-induced reductions in $PaCO_2$ (i.e., respiratory alkalosis) correspond with the slow exchange of CSF [HCO_3^-] to normalize CSF pH and CBF with progressive acclimatization, further substantiating that arterial pH *per se* does not dictate CBF regulation. We interpret the unaltered CBF-$PaCO_2$ response between trials at HA to indicate that CBF is acutely regulated by $PaCO_2$ within the context of acute and chronic alterations in arterial pH following partial acclimatization to 5,050 m.

Experimental considerations: With this experimental protocol we were restricted by the lasting effects of ACZ and $NaHCO_3^-$ on acid-base balance; therefore, the order of trials were not randomized within altitudes. Without an appropriate time-control, changes in CaO_2 may have occurred due to acclimatization to HA throughout the testing sessions that lasted two days following 14-20 days at 5,050 m; however, this is unlikely as we observed a related increase in CaO_2 with ACZ at SL. It is noteworthy that ACZ attenuated MAP at both altitudes; e.g., MAP was reduced by approximately 12% at HA (i.e., Δ -13 ± 5 mmHg; **Table 2**), likely indicating a direct vasodilatory influence of ACZ on the systemic circulation (Parati *et al.*, 2013; Eskandari *et al.*, 2018) via opening of Ca^{2+}-activated K^+ channels (Pickkers *et al.*, 2001). Notwithstanding, covariate analysis revealed no influence of resting MAP on *g*CBF within each trial and the resting $gCBF_{CVC}$ response was unaffected by ACZ at both altitudes (**Table 2**). Previous reports indicate that cerebral oxidative metabolism is higher at rest following 4-6 days at 5,050 m (Smith *et al.*, 2014), unaltered following 5 weeks at 5,260 m (Møller *et al.*, 2002), and likely varies with acute alterations in $PaCO_2$ (Willie *et al.*, 2015). As such, future investigations on the relationship between cerebrovascular acid-base regulation and metabolism at high-altitude are merited. It is unknown whether ACZ may differentially affect cerebral oxidative metabolism at high-altitude.

Technical considerations: A strength of the current study was the use of regional volumetric Q_{ICA} and Q_{VA} to calculate *g*CBF at rest; however, such an approach would be preferable to MCA*v*/PCA*v* estimates of CBF throughout the cerebrovascular CO_2 reactivity tests. Duplex ultrasound facilitates B-mode arterial diameter in the sagittal axis and pulse-wave blood velocity measurements to concurrently calculate volumetric blood flow (Thomas *et al.*, 2015).

Transcranial Doppler ultrasound only provides pulse-wave velocity without brightness (B-mode) imaging; therefore, this technique relies on the assumptions that arterial diameter and insonation angle are not changing (Ainslie & Hoiland, 2014). We appreciate that MCA*v* systematically underestimates Duplex ultrasound and Fick-derived CBF reactivity to $PaCO_2$ at SL and 5,050 m (Willie *et al.*, 2015). As such, we attempted to standardize the response between trials at each altitude by utilizing an approach to calculate relative changes in CBV.

Additionally, continuous arterial blood gas sampling throughout the CO_2 reactivity tests at SL would have been advantageous to directly ascertain the changes in buffering capacity with respiratory acidosis/alkalosis. Bland-Altman analysis revealed that the resting $P_{ET}CO_2$-$PaCO_2$ gradients at SL and HA were 1.0 ± 3.1 and 3.7 ± 1.6 mmHg, respectively; consistent with previously reported values (Willie *et al.*, 2012; Tymko *et al.*, 2015). Importantly, the linearly related $P_{ET}CO_2$-$PaCO_2$ gradient (R^2 0.94, $P < 0.001$) throughout the cerebrovascular CO_2 reactivity tests at HA was consistent with the gradient observed at rest (e.g., 3.7 ± 1.6 vs. 3.7 ± 1.7 mmHg); therefore, the small (<3 mmHg) $P_{ET}CO_2$-$PaCO_2$ gradient at HA versus SL likely did not alter our findings. Relatedly, the validity of arterial pH and acid-base buffering as an index of CSF pH changes at HA deserves consideration. Reductions in CSF [HCO_3^-] follow changes in arterial blood indicating that passive exchange of CO_2 across the blood-brain-barrier and resultant re-equilibrium of the reaction between CO_2 and HCO_3^- provokes changes in CSF [HCO_3^-] and pH (Forster *et al.*, 1975; Weiskopf *et al.*, 1976). Dempsey and colleagues (1974) reported consistent CSF to arterial pH gradients (Δ -0.08 pH units) and closely matched [HCO_3^-] between CSF and arterial samples at SL and following 3-4 weeks at 3,100 m. Additionally, they reported that the relative partial metabolic/respiratory compensation with acclimatization to HA was not different between arterial blood and CSF with respect to [H^+]/pH and PCO_2 changes; as such, these data support the efficacy of arterial acid-base changes as an index of CSF regulation.

CONCLUSION

These findings reveal that in the context of acute and chronic changes in arterial pH – via partial acclimatization to high-altitude and experimentally controlled metabolic acidosis/alkalosis – including within trial acute alterations in $PaCO_2$ (i.e., respiratory acidosis/alkalosis), the CBF response is consistent with changes in $PaCO_2$ rather than the prevailing arterial [H^+]/pH *per se*.

In support of this, we show that resting CBF and the cerebrovascular reactivity to $PaCO_2$ were unchanged between trials within each altitude even though arterial pH and $[HCO_3^-]$ (i.e., buffering capacity) were effectively altered. Taken together, these findings are consistent with previous studies indicating $PaCO_2$ and resultant passive diffusion of CO_2 across the vascular wall to alter perivascular pH, rather than arterial pH *per se*, acutely regulates CBF in humans.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing Interests

None to declare.

Author Contributions

PNA, SJEL, MS, and KRB conceived and designed the research. KJS, NL, RLH, CKW, SJEL, MS, KRB, DBM, and PNA acquired the data. HGC and RLH analyzed the data. HGC, RLH, and PNA interpreted the data. HGC drafted the manuscript. All authors revised the manuscript and provided intellectual feedback and agree to be accountable for all aspects of the work.

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TABLE CAPTIONS

Table 1. Arterial blood gas and acid-base parameters at rest during control, acetazolamide, and sodium bicarbonate infusion conditions at sea level (344 m) and high-altitude (5,050 m)

Abbreviations: Hydrogen concentration, $[H^+]$; bicarbonate concentration, $[HCO_3^-]$; arterial carbon dioxide tension, $PaCO_2$; arterial oxygen tension, PaO_2; arterial oxygen saturation, SaO_2; arterial oxygen content, CaO_2; hemoglobin concentration, [Hb]. Trial main effect pairwise comparisons: $^{a}P < 0.05$ versus control; $^{b}P < 0.05$ versus ACZ. Trial × altitude interaction pairwise comparisons: $^{\#}P < 0.05$ versus control within altitude; $^{\$}P < 0.05$ versus ACZ within altitude; $^{+}P < 0.05$ versus control between altitudes; $^{\%}P < 0.05$ versus ACZ between altitudes; $^{\&}P < 0.05$ versus ACZ+HCO_3^- between altitudes. Data are mean ± SD. Sample sizes: n=7 for all three trials at sea level (SL), n=10 for the control and ACZ trials at high-altitude (HA), and n=5 for the ACZ+HCO_3^- trial at HA.

Table 2. Cardiorespiratory and cerebrovascular parameters at rest during control, acetazolamide, and sodium bicarbonate infusion conditions at sea level (344 m) and high-altitude (5,050 m)

Abbreviations: Ventilation, $\dot{V}_E$; tidal volume, V_T; respiratory frequency, f_R; breaths per minute, BPM; heart rate, HR; beats per minute, bpm; mean arterial pressure, MAP; cerebral oxygen delivery, CDO_2; global cerebral blood flow, *g*CBF; global cerebrovascular conductance, $gCBF_{CVC}$. Trial main effect pairwise comparisons: $^{b}P < 0.05$ versus ACZ. Trial × altitude interaction pairwise comparisons: $^{\%}P < 0.05$ versus ACZ between altitudes. Data are mean ± SD for n=11 at sea level (SL) and n=10 at high-altitude (HA).

Table 3. Relative cerebrovascular CO_2 reactivity during control, acetazolamide, and sodium bicarbonate infusion conditions at sea level (344 m) and high-altitude (5,050 m)

Abbreviations: Middle cerebral artery blood velocity, MCA*v*; Posterior cerebral artery blood velocity, PCA*v*; cerebrovascular conductance, CVC; hypocapnia, hypo-; hypercapnia, hyper-. Trial main effect pairwise comparisons: $^{a}P < 0.05$ versus control. Trial × altitude interaction pairwise comparisons: $^{\#}P < 0.05$ versus control within altitude; $^{\$}P < 0.05$ versus ACZ within altitude; $^{+}P < 0.05$ versus control between altitudes; $^{\%}P < 0.05$ versus ACZ between altitudes; $^{\&}P < 0.05$ versus ACZ+HCO_3^- between altitudes. Data are mean ± SD. Sample sizes: SL control, n=10; SL ACZ, n=11; SL ACZ+HCO_3^-, n=11; HA control, n=10; HA ACZ, n=9; HA ACZ+HCO_3^-, n=9.

Table 4. Absolute cerebrovascular CO_2 reactivity during control, acetazolamide, and sodium bicarbonate infusion conditions at sea level (344 m) and high-altitude (5,050 m)

Abbreviations: Middle cerebral artery blood velocity, MCA*v*; Posterior cerebral artery blood velocity, PCA*v*; cerebrovascular conductance, CVC; hypocapnia, hypo-; hypercapnia, hyper-. Trial main effect pairwise comparisons: $^{a}P < 0.05$ versus control. Trial × altitude interaction pairwise comparisons: $^{\#}P < 0.05$ versus control within altitude; $^{\$}P < 0.05$ versus ACZ within

altitude; $^{+}P < 0.05$ versus control between altitudes; $^{\&}P < 0.05$ versus ACZ+HCO_3^- between altitudes. Data are mean ± SD. Sample sizes: SL control, n=10; SL ACZ, n=11; SL ACZ+HCO_3^-, n=11; HA control, n=10; HA ACZ, n=9; HA ACZ+HCO_3^-, n=9.

FIGURE CAPTIONS

Figure 1. Global cerebral blood flow (*g*CBF) regulation at rest during acetazolamide (ACZ) and acetazolamide with bicarbonate (ACZ+HCO_3^-) trials at sea level (**A** & **C**) and high-altitude (**B** & **D**). Each panel shows the intra-individual variability between the absolute change in arterial H^+ (**A** & **B**) and PCO_2 ($PaCO_2$) (**C** & **D**) and the respective relative change in *g*CBF. The absolute change in H^+ and $PaCO_2$, and the relative change in *g*CBF are compared to the control trial values, respectively. Overall, an unchanged *g*CBF response corresponded with a higher arterial [H^+] (i.e., lower pH) as well as a reduction in $PaCO_2$ following ACZ; that is, respiratory compensation (i.e., Δ $PaCO_2$), rather than the prevailing arterial [H^+]/pH, is sufficient to offset any changes in *g*CBF with ACZ and ACZ+HCO_3^- acid-base alterations at SL and HA. Data are individual values with group averages. Sample sizes: n=8 for SL both trials, n=8 for ACZ at HA, and n=5 for ACZ+HCO_3^- at HA.

Figure 2. Acid-base balance and cerebrovascular regulation throughout CO_2 reactivity tests during control, acetazolamide (ACZ) and acetazolamide with bicarbonate (ACZ+HCO_3^-) trials at high-altitude. There was a significant leftward shift in the x-intercept in the absolute MCA*v* versus pH response within the ACZ versus control trial (7.59 ± 0.04 vs. 7.71 ± 0.08, P = 0.002); however, this was reversed with ACZ+HCO_3^- (7.65 ± 0.04 vs. 7.71 ± 0.08, P = 0.086) (**A**). These leftward x-intercept shifts were consistent with the $PaCO_2$ versus pH response between trials (**C**); i.e., the altered relationship between $PaCO_2$-pH was reflected in a leftward shift in the MCA*v*-pH response. It is noteworthy that the absolute MCA*v* was not different at each stage of $PaCO_2$ between trials at HA (**B**). Throughout the CO_2 reactivity tests, CaO_2 was not significantly different between trials when indexed against arterial pH (**D**). Data are mean ± SD for n=10 for control & ACZ and n=5 for ACZ+HCO_3^-.

Figure 3. Cerebrovascular regulation throughout CO_2 reactivity tests during control, acetazolamide (ACZ) and acetazolamide with bicarbonate (ACZ+HCO_3^-) trials at sea level (SL) and high-altitude (HA). At SL, the MCA_{CVC}-$P_{ET}CO_2$ response was leftward shifted with ACZ and ACZ+HCO_3^- and this was likely explained by the significant reduction in *resting* $PaCO_2$ within these trials (**A**). As the absolute change in resting $PaCO_2$ with ACZ and ACZ+HCO_3^- was less at HA, there was no difference between the MCA_{CVC}-$PaCO_2$ responses between trials (**B**). The MCA*v* hypo- and hypercapnic CVR was consistently higher at HA compared to SL irrespective of trial (**C** & **D**). Across altitudes, MCA*v* hypercapnic CVR was higher during the control trial than ACZ and ACZ+HCO_3^- with no influence of altitude *per se* (**D**). Data are mean ± SD (**A** & **B**) and individual values with group averages (**C** & **D**). Sample sizes: (**A**) n=10 for control and n=11 for ACZ & ACZ+HCO_3^-; (**B**) n=10 for control & ACZ and n=5 for ACZ+HCO_3^-; (**C**) & (**D**) n=10 for SL control, n=11 for SL ACZ & ACZ+HCO_3^-, n=10 for HA control, n=9 for HA ACZ & ACZ+HCO_3^-.

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Table 1. Arterial blood gas and acid-base parameters at rest during control, acetazolamide, and sodium bicarbonate infusion conditions at sea level (344 m) and high-altitude (5,050 m)

		Control	ACZ	ACZ+HCO_3^-	*P-Values*		
					Trial	*Altitude*	*Trial × Altitude*
pH	SL	7.42 ± 0.01^{b}	7.36 ± 0.03	7.44 ± 0.02^{b}	**P < 0.001**	**P = 0.002**	P = 0.117
	HA	7.47 ± 0.07^{b}	7.40 ± 0.02	7.44 ± 0.02^{b}			
$[H^+]$ (nM)	SL	37.9 ± 1.3^{b}	44.0 ± 3.2	36.6 ± 2.1^{b}	**P < 0.001**	**P = 0.005**	P = 0.172
	HA	34.4 ± 6.8^{b}	39.8 ± 2.1	36.0 ± 1.6^{b}			
$[HCO_3^-]$ ($mEq \cdot l^{-1}$)	SL	$25.5 \pm 1.6^{\$,+}$	$19.2 \pm 0.9^{a,\%}$	$24.6 \pm 1.2^{a,b,\$,\&}$	**P < 0.001**	**P < 0.001**	**P = 0.002**
	HA	$17.4 \pm 2.5^{\$,+}$	$13.5 \pm 1.3^{a,\%}$	$15.9 \pm 1.2^{a,b,\$,\&}$			
Base Excess ($mEq \cdot l^{-1}$)	SL	$1.3 \pm 1.4^{b,\$,+}$	$-5.4 \pm 0.8^{\%}$	$0.7 \pm 1.2^{b,\$,\&}$	**P < 0.001**	**P < 0.001**	**P = 0.025**
	HA	$-6.3 \pm 3.6^{b,\$,+}$	$-11.3 \pm 1.4^{\%}$	$-8.2 \pm 1.2^{b,\$,\&}$			
$PaCO_2$ (mmHg)	SL	40.1 ± 3.6	35.2 ± 3.5^{a}	37.1 ± 2.9	**P < 0.001**	**P < 0.001**	P = 0.073
	HA	23.7 ± 1.8	21.7 ± 2.1^{a}	23.2 ± 2.2			
PaO_2 (mmHg)	SL	94 ± 13	102 ± 7^{a}	98 ± 9	**P = 0.005**	**P < 0.001**	P = 0.703
	HA	43 ± 2	48 ± 2^{a}	46 ± 3			
SaO_2 (%)	SL	$97.0 \pm 1.6^{+}$	$97.8 \pm 0.5^{a,\%}$	$97.5 \pm 0.7^{a,\&}$	**P < 0.001**	**P < 0.001**	**P = 0.038**
	HA	$82.7 \pm 2.2^{+}$	$85.0 \pm 2.2^{a,\#,\%}$	$85.0 \pm 2.4^{a,\#,\&}$			
CaO_2 ($mL \cdot dL^{-1}$)	SL	$19.8 \pm 0.7^{\$,+}$	$21.0 \pm 0.5^{a,\%}$	$19.7 \pm 1.0^{a,b,\$}$	**P < 0.001**	**P < 0.001**	**P = 0.003**
	HA	$17.5 \pm 1.1^{+}$	$19.3 \pm 1.2^{a,\#,\%}$	$19.1 \pm 0.5^{a,b,\#}$			
[Hb] ($g \cdot dL^{-1}$)	SL	$15.0 \pm 0.7^{\$,+}$	$15.8 \pm 0.4^{a,\%}$	$14.9 \pm 0.8^{a,b,\$,\&}$	**P < 0.001**	**P < 0.001**	**P = 0.024**
	HA	$15.7 \pm 1.2^{+}$	$16.9 \pm 1.1^{a,\#,\%}$	$16.6 \pm 0.9^{a,b,\#,\&}$			

Abbreviations: Hydrogen concentration, $[H^+]$; bicarbonate concentration, $[HCO_3^-]$; arterial carbon dioxide tension, $PaCO_2$; arterial oxygen tension, PaO_2; arterial oxygen saturation, SaO_2; arterial oxygen content, CaO_2; hemoglobin concentration, [Hb]. Trial main effect pairwise comparisons: $^{a}P < 0.05$ versus control; $^{b}P < 0.05$ versus ACZ. Trial × altitude interaction pairwise comparisons: $^{\#}P < 0.05$ versus control within altitude; $^{\$}P < 0.05$ versus ACZ within altitude; $^{+}P < 0.05$ versus control between altitudes; $^{\%}P < 0.05$ versus ACZ between altitudes; $^{\&}P < 0.05$ versus ACZ+HCO_3^- between altitudes. Data are mean ± SD. Sample sizes: n=7 for all three trials at sea level (SL), n=10 for the control and ACZ trials at high-altitude (HA), and n=5 for the ACZ+HCO_3^- trial at HA.

Table 2. Cardiorespiratory and cerebrovascular parameters at rest during control, acetazolamide, and sodium bicarbonate infusion conditions at sea level (344 m) and high-altitude (5,050 m)

		Control	ACZ	ACZ+HCO_3^-	*P-Values*		
					Trial	*Altitude*	*Trial × Altitude*
$\dot{V}_E$ ($L \cdot min^{-1}$)	SL	11.5 ± 2.2	11.8 ± 1.4	12.0 ± 1.3	P = 0.434	**P < 0.001**	P = 0.813
	HA	17.6 ± 3.7	19.1 ± 1.5	18.7 ± 3.6			
V_T (L)	SL	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	P = 0.499	**P < 0.001**	P = 0.085
	HA	1.2 ± 0.4	1.1 ± 0.1	1.3 ± 0.2			
f_R (BPM)	SL	14 ± 3	14 ± 2[%]	16 ± 2	P = 0.663	**P = 0.026**	**P = 0.046**
	HA	16 ± 5	18 ± 2[%]	15 ± 2			
HR (bpm)	SL	59 ± 6	60 ± 9	60 ± 10	P = 0.126	**P < 0.001**	P = 0.066
	HA	72 ± 12	76 ± 14	70 ± 14			
MAP (mmHg)	SL	86 ± 8[b]	82 ± 9	85 ± 11[b]	**P = 0.004**	**P < 0.001**	P = 0.083
	HA	105 ± 7[b]	92 ± 7	102 ± 7[b]			
CDO_2 ($mL \cdot min^{-1}$)	SL	88 ± 43	89 ± 31	91 ± 42	P = 0.678	**P = 0.009**	P = 0.627
	HA	120 ± 43	125 ± 38	108 ± 13			
*g*CBF ($mL \cdot min^{-1}$)	SL	450 ± 204	423 ± 152	460 ± 204	P = 0.356	**P < 0.001**	P = 0.295
	HA	683 ± 225	647 ± 189	607 ± 120			
$gCBF_{CVC}$ ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	SL	5.38 ± 2.25	5.11 ± 1.77	5.45 ± 2.28	P = 0.583	**P = 0.022**	P = 0.218
	HA	6.54 ± 2.02	7.15 ± 1.88	5.92 ± 0.99			

Abbreviations: Ventilation, $\dot{V}_E$; tidal volume, V_T; respiratory frequency, f_R; breaths per minute, BPM; heart rate, HR; beats per minute, bpm; mean arterial pressure, MAP; cerebral oxygen delivery, CDO_2; global cerebral blood flow, *g*CBF; global cerebrovascular conductance, $gCBF_{CVC}$. Trial main effect pairwise comparisons: [b]P < 0.05 versus ACZ. Trial × altitude interaction pairwise comparisons: [%]P < 0.05 versus ACZ between altitudes. Data are mean ± SD for n=11 at sea level (SL) and n=10 at high-altitude (HA).

Table 3. Relative cerebrovascular CO_2 reactivity during control, acetazolamide, and sodium bicarbonate infusion conditions at sea level (344 m) and high-altitude (5,050 m)

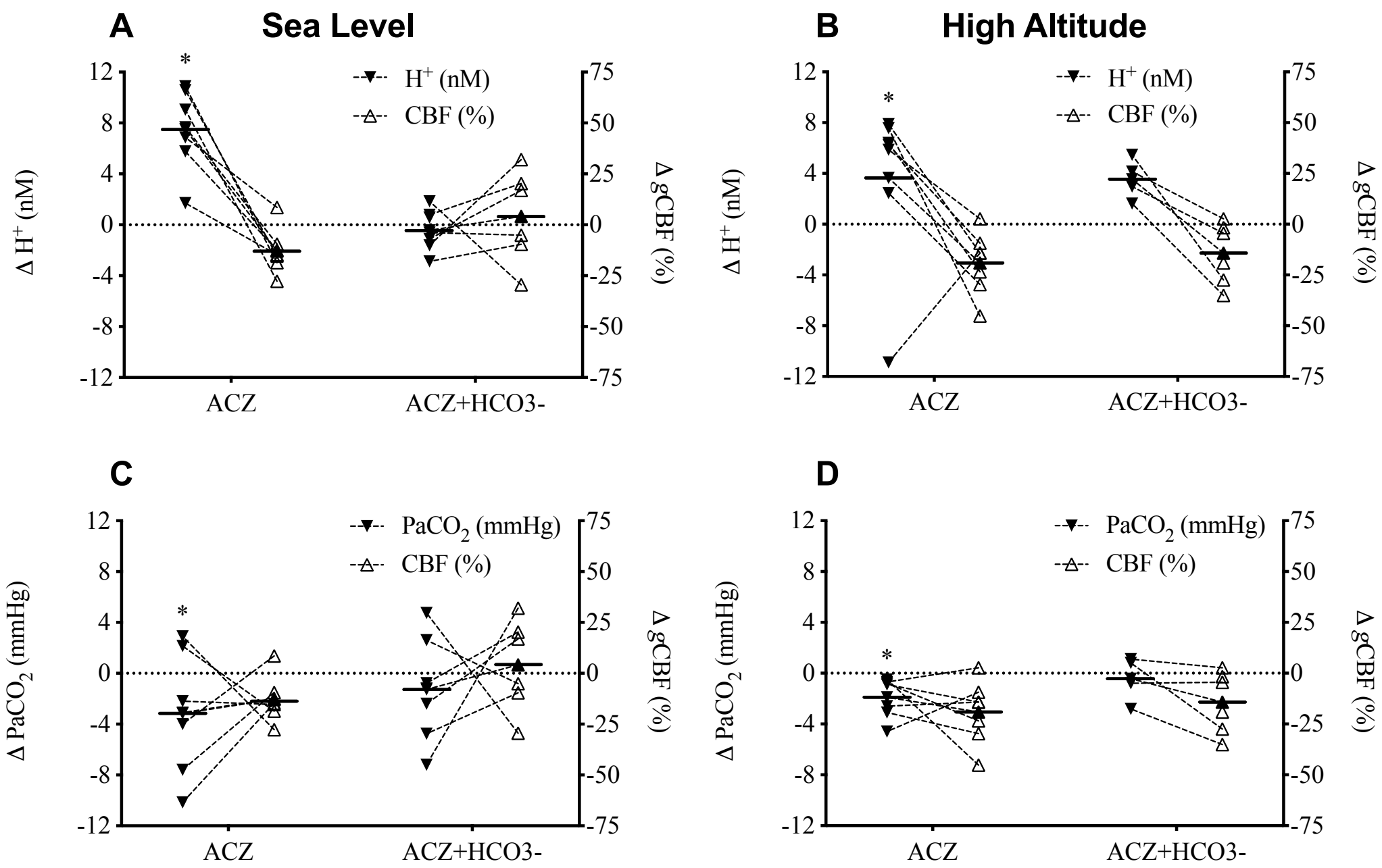
		Control	ACZ	ACZ+HCO_3^-	*P-Values*		
Reactivity (Δ% per Δ mmHg)					*Trial*	*Altitude*	*Trial × Altitude*
MCA*v* hypo-	SL	3.0 ± 0.5	2.9 ± 0.4	3.3 ± 0.5	P = 0.276	**P < 0.001**	P = 0.413
	HA	4.2 ± 0.8	4.0 ± 0.5	4.2 ± 0.5			
MCA*v* hyper-	SL	4.7 ± 1.1	3.7 ± 1.1[a]	3.2 ± 1.0[a]	**P = 0.005**	**P < 0.001**	P = 0.424
	HA	7.1 ± 1.6	6.0 ± 1.0[a]	6.4 ± 1.5[a]			
MCA_{CVC} hypo-	SL	2.9 ± 0.5[+]	2.8 ± 0.3[%]	3.2 ± 0.6	P = 0.539	**P < 0.001**	**P = 0.045**
	HA	3.7 ± 0.7[+]	3.9 ± 0.4[%]	3.6 ± 0.6			
MCA_{CVC} hyper-	SL	2.8 ± 1.0	2.5 ± 0.7	2.1 ± 0.7[&]	P = 0.252	**P = 0.018**	**P = 0.030**
	HA	3.1 ± 1.2	2.6 ± 0.7	3.4 ± 1.0[&]			
PCA*v* hypo-	SL	2.7 ± 0.3[+]	3.0 ± 0.5[%]	3.2 ± 0.8[&]	P = 0.805	**P < 0.001**	**P = 0.006**
	HA	4.4 ± 0.8[+]	4.2 ± 0.7[%]	3.9 ± 0.5[&]			
PCA*v* hyper-	SL	4.1 ± 1.2	3.9 ± 1.5	3.3 ± 1.2	P = 0.073	**P < 0.001**	P = 0.470
	HA	7.9 ± 2.2	6.7 ± 0.8	6.6 ± 2.2			
PCA_{CVC} hypo-	SL	2.6 ± 0.3[+]	2.9 ± 0.5[%]	3.1 ± 0.8	P = 0.081	**P < 0.001**	**P = 0.001**
	HA	3.9 ± 0.7[+]	4.1 ± 0.5[%]	3.2 ± 0.7[#,$]			
PCA_{CVC} hyper-	SL	2.4 ± 1.1	2.7 ± 1.2	2.2 ± 0.9	P = 0.849	**P = 0.002**	P = 0.418
	HA	3.7 ± 1.6	3.1 ± 0.6	3.4 ± 1.4			

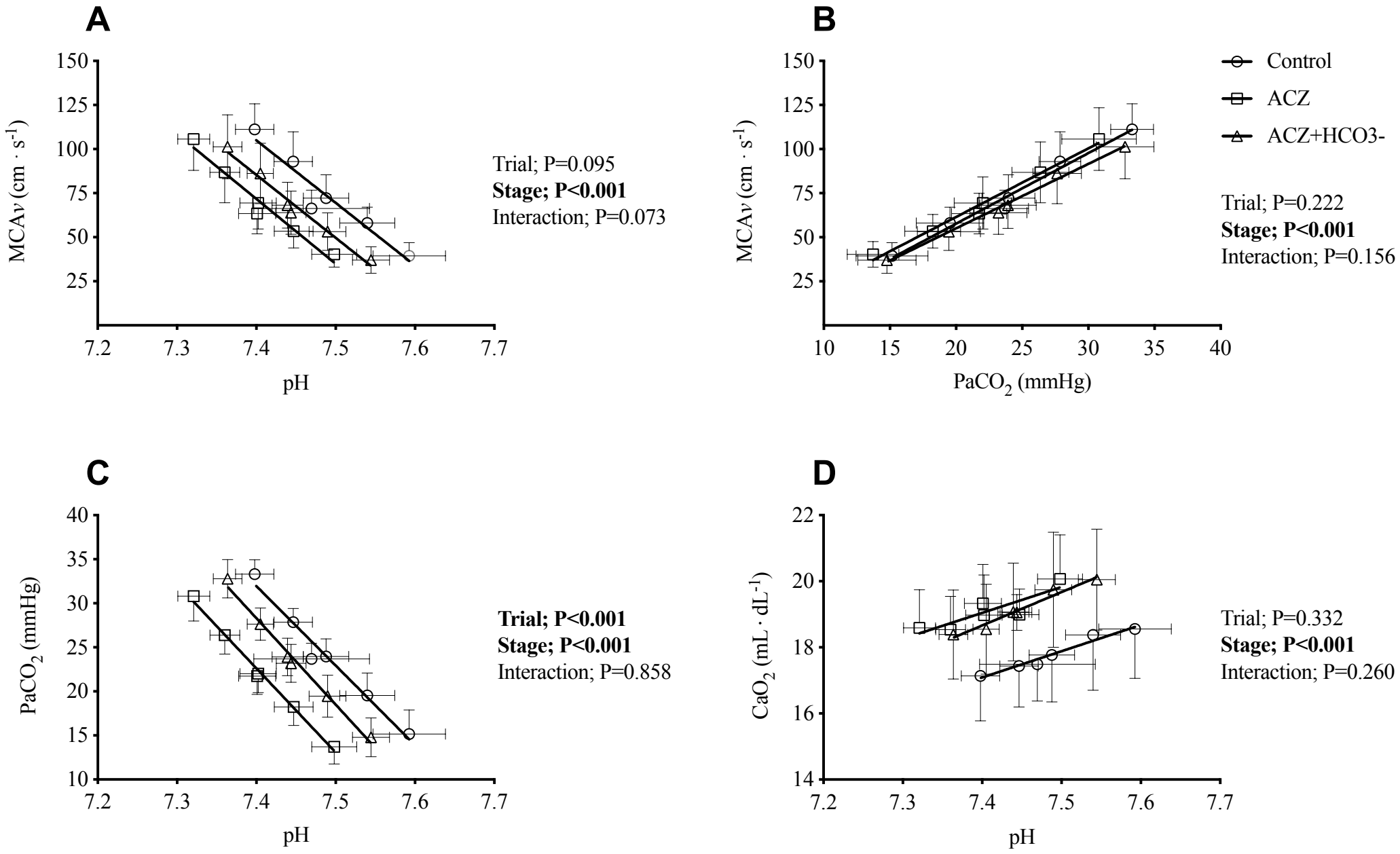
Abbreviations: Middle cerebral artery blood velocity, MCA*v*; Posterior cerebral artery blood velocity, PCA*v*; cerebrovascular conductance, CVC; hypocapnia, hypo-; hypercapnia, hyper-. Trial main effect pairwise comparisons: [a]P < 0.05 versus control. Trial × altitude interaction pairwise comparisons: [#]P < 0.05 versus control within altitude; [$]P < 0.05 versus ACZ within altitude; [+]P < 0.05 versus control between altitudes; [%]P < 0.05 versus ACZ between altitudes; [&]P < 0.05 versus ACZ+HCO_3^- between altitudes. Data are mean ± SD. Sample sizes: SL control, n=10; SL ACZ, n=11; SL ACZ+HCO_3^-, n=11; HA control, n=10; HA ACZ, n=9; HA ACZ+HCO_3^-, n=9.

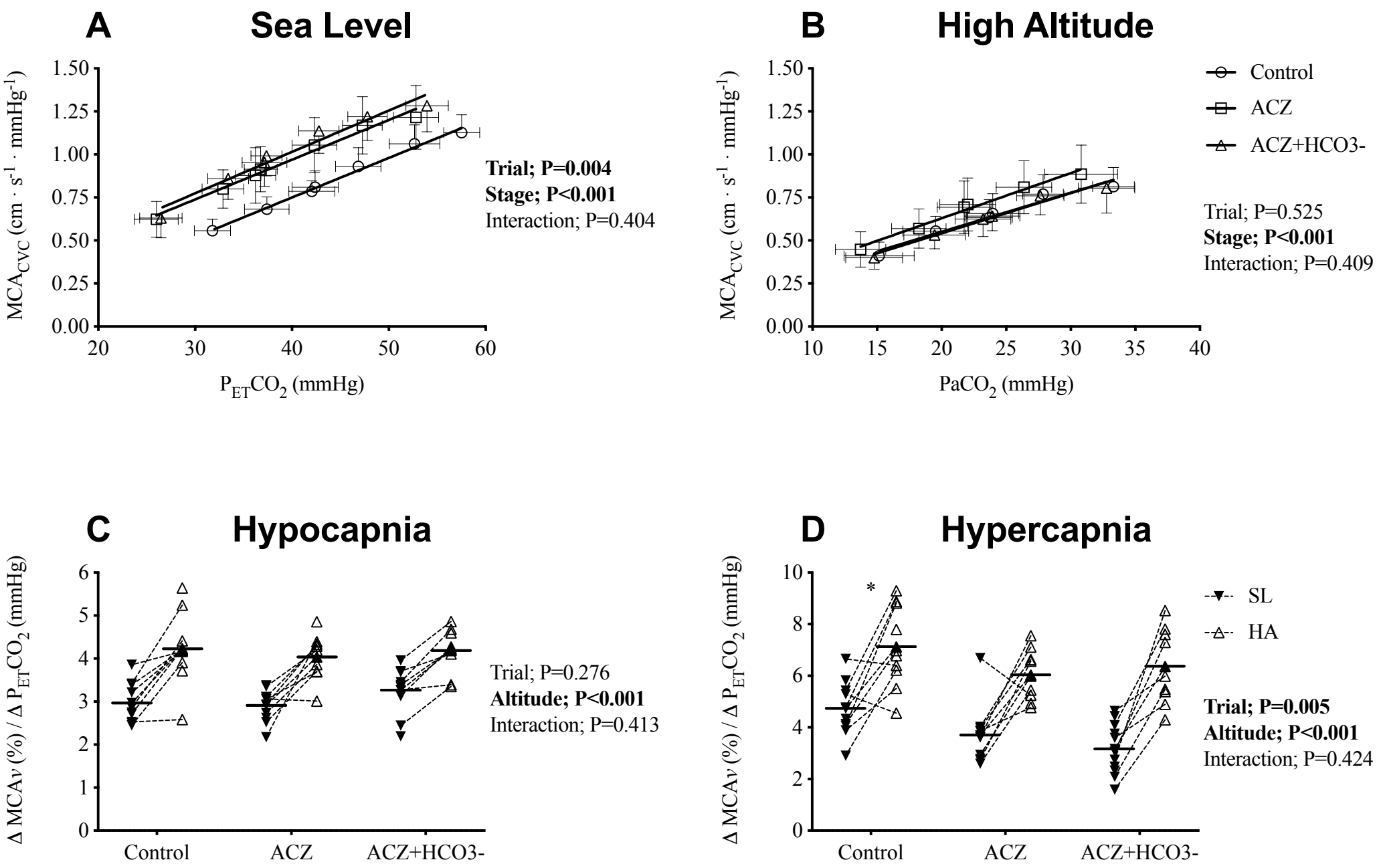
Table 4. Absolute cerebrovascular CO_2 reactivity during control, acetazolamide, and sodium bicarbonate infusion conditions at sea level (344 m) and high-altitude (5,050 m)

		Control	ACZ	ACZ+HCO_3^-	*P-Values*		
Reactivity (Δ cm/s per Δ mmHg)					*Trial*	*Altitude*	*Trial × Altitude*
MCA*v* hypo-	SL	2.0 ± 0.4^{+}	2.1 ± 0.4	2.7 ± 0.7$^{\#,\$}$	P = 0.073	**P = 0.028**	**P = 0.016**
	HA	2.8 ± 0.8^{+}	2.6 ± 0.7	2.7 ± 0.6			
MCA*v* hyper-	SL	3.1 ± 0.7	2.6 ± 0.8^{a}	2.5 ± 0.6^{a}	**P = 0.004**	**P < 0.001**	P = 0.699
	HA	4.7 ± 1.1	3.8 ± 0.7^{a}	3.6 ± 1.1^{a}			
MCA_{CVC} hypo-	SL	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.01$^{\#,\&}$	P = 0.081	P = 0.127	**P = 0.012**
	HA	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01$^{\&}$			
MCA_{CVC} hyper-	SL	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	P = 0.498	**P = 0.037**	P = 0.911
	HA	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01			
PCA*v* hypo-	SL	1.3 ± 0.5	1.4 ± 0.4	1.7 ± 0.7	P = 0.447	**P = 0.017**	P = 0.056
	HA	2.0 ± 0.5	1.8 ± 0.5	1.8 ± 0.5			
PCA*v* hyper-	SL	2.0 ± 0.8	1.8 ± 0.7	1.7 ± 0.6	P = 0.084	**P < 0.001**	P = 0.427
	HA	3.6 ± 1.1	2.9 ± 0.8	2.9 ± 0.7			
PCA_{CVC} hypo-	SL	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.01$^{\#,\&}$	P = 0.168	P = 0.385	**P = 0.015**
	HA	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.00$^{\&}$			
PCA_{CVC} hyper-	SL	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	P = 0.940	P = 0.199	P = 0.577
	HA	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.00			

Abbreviations: Middle cerebral artery blood velocity, MCA*v*; Posterior cerebral artery blood velocity, PCA*v*; cerebrovascular conductance, CVC; hypocapnia, hypo-; hypercapnia, hyper-. Trial main effect pairwise comparisons: $^{a}P < 0.05$ versus control. Trial × altitude interaction pairwise comparisons: $^{\#}P < 0.05$ versus control within altitude; $^{\$}P < 0.05$ versus ACZ within altitude; $^{+}P < 0.05$ versus control between altitudes; $^{\&}P < 0.05$ versus ACZ+HCO_3^- between altitudes. Data are mean ± SD. Sample sizes: SL control, n=10; SL ACZ, n=11; SL ACZ+HCO_3^-, n=11; HA control, n=10; HA ACZ, n=9; HA ACZ+HCO_3^-, n=9.


A
Sea Level
B
High Altitude
C
D
H+ (nM)
CBF (%)
PaCO2 (mmHg)
Δ H+ (nM)
Δ PaCO2 (mmHg)
Δ gCBF (%)
ACZ
ACZ+HCO3-
12
8
4
0
-4
-8
-12
75
50
25
0
-25
-50
-75
*


A
MCAv (cm · s-1)
150
125
100
75
50
25
7.2
7.3
7.4
7.5
7.6
7.7
pH
Trial; P=0.095
Stage; P<0.001
Interaction; P=0.073
B
MCAv (cm · s-1)
10
15
20
25
30
35
40
PaCO2 (mmHg)
Control
ACZ
ACZ+HCO3-
Trial; P=0.222
Stage; P<0.001
Interaction; P=0.156
C
PaCO2 (mmHg)
40
35
30
25
20
15
10
pH
Trial; P<0.001
Stage; P<0.001
Interaction; P=0.858
D
CaO2 (mL · dL-1)
22
20
18
16
14
pH
Trial; P=0.332
Stage; P<0.001
Interaction; P=0.260


A
Sea Level
MCA$_{CVC}$ (cm · s^{-1} · mmHg^{-1})
1.50
1.25
1.00
0.75
0.50
0.25
0.00
20
30
40
50
60
P$_{ET}$CO$_2$ (mmHg)
Trial; P=0.004
Stage; P<0.001
Interaction; P=0.404
B
High Altitude
MCA$_{CVC}$ (cm · s^{-1} · mmHg^{-1})
10
15
20
25
30
35
40
PaCO$_2$ (mmHg)
Control
ACZ
ACZ+HCO3-
Trial; P=0.525
Stage; P<0.001
Interaction; P=0.409
C
Hypocapnia
Δ MCAv (%) / Δ P$_{ET}$CO$_2$ (mmHg)
6
5
4
3
2
1
0
Control
ACZ
ACZ+HCO3-
Trial; P=0.276
Altitude; P<0.001
Interaction; P=0.413
D
Hypercapnia
Δ MCAv (%) / Δ P$_{ET}$CO$_2$ (mmHg)
10
8
6
4
2
0
*
Control
ACZ
ACZ+HCO3-
SL
HA
Trial; P=0.005
Altitude; P<0.001
Interaction; P=0.424