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Regulation of cerebral blood flow by arterial PCO₂ independent of metabolic acidosis at 5,050 m

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Title: Regulation of cerebral blood flow by arterial PCO2 independent of metabolic acidosis at 5,050 m

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Ryan Hoiland: Acquisition or analysis or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work Christopher Willie: Acquisition or analysis or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work Samuel Lucas: Conception or design of the work; Acquisition or analysis or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work Mike Stembridge: Conception or design of the work; Acquisition or analysis or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work Keith Burgess: Conception or design of the work; Acquisition or analysis or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work David MacLeod: Acquisition or analysis or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work Philip Ainslie: Conception or design of the work; Acquisition or analysis or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work

Running Title: ACID-BASE BALANCE AND CEREBRAL BLOOD FLOW AT HIGH-ALTITUDE

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1	Regulation of cerebral blood flow by arterial PCO ₂ independent of metabolic acidosis at 5,050 m
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28 Summary:

- We investigated the influence of arterial PCO₂ (PaCO₂) 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 PaCO₂ following
 metabolic acidosis (via two days of oral acetazolamide; ACZ) with and without acute
 restoration of pH (via intravenous sodium bicarbonate; ACZ+HCO₃⁻).
- Total resting CBF was unchanged between trials within each altitude even though arterial
 pH and [HCO₃⁻] (i.e., buffering capacity) were effectively altered.
- The cerebrovascular responses to changes in arterial [H⁺]/pH were consistent with the
 altered relationship between PaCO₂ 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 PaCO₂ was
 unchanged between trials at high-altitude, indicating that CBF is acutely regulated by
 PaCO₂ rather than *arterial* pH.
- 43
- 44 Key Words: Cerebral blood flow, acid-base balance, high-altitude, CO₂ reactivity,
- 45 acetazolamide, sodium bicarbonate, metabolic acidosis

46 ABSTRACT

47 Alterations in acid-base balance with progressive acclimatization to high-altitude have been 48 well-established; however, how respiratory alkalosis and resultant metabolic compensation 49 interact to regulate cerebral blood flow (CBF) is uncertain. We addressed this via three separate 50 experimental trials at sea level and following partial acclimatization (14 to 20 days) at 5,050 m; 51 involving: 1) resting acid-base balance (control); 2) following metabolic acidosis via two days of 52 oral acetazolamide at 250 mg every 8 hours (ACZ; pH: Δ -0.07±0.04 and base excess: Δ -5.7±1.9 mEq·1⁻¹, trial effects: P<0.001 and P<0.001, respectively); and 3) after acute normalization of 53 54 arterial acidosis via intravenous sodium bicarbonate (ACZ+HCO₃⁻; pH: Δ -0.01±0.04 and base excess: $\Delta -1.5 \pm 2.1 \text{ mEq} \cdot l^{-1}$, trial effects: P=1.000 and P=0.052, respectively). Within each trial, 55 56 we utilized transcranial Doppler ultrasound to assess the cerebral blood velocity (CBV) response to stepwise alterations in arterial PCO_2 (PaCO₂); i.e., cerebrovascular CO_2 reactivity. Resting 57 58 CBF (via Duplex ultrasound) was unaltered between trials within each altitude, indicating that 59 respiratory compensation (i.e., Δ -3.4±2.3 mmHg PaCO₂, trial effect: P<0.001) was sufficient to 60 offset any elevations in CBF induced via the ACZ-mediated metabolic acidosis. Between trials at 61 high-altitude, we observed consistent *leftward* shifts in both the PaCO₂-pH and CBV-pH responses across the CO₂ reactivity tests with experimentally *reduced* arterial pH via ACZ. 62 When indexed against PaCO₂ - rather than pH - the absolute CBV and sensitivity of CBV-63 64 PaCO₂ was unchanged between trials at high-altitude. Taken together, following acclimatization, CO₂-mediated changes in cerebrovascular tone rather than *arterial* [H⁺]/pH is integral to CBF 65 66 regulation at high-altitude.

67

68 Word Count: 250/250

69 INTRODUCTION

70

71 The cerebral vasculature is exceptionally sensitive to alterations in the partial pressure of arterial 72 carbon dioxide (PaCO₂) (Hoiland et al., 2019) such that increases and decreases in PaCO₂ (i.e., 73 hyper- and hypocapnia) rapidly increase and decrease cerebral blood flow (CBF), respectively 74 (Kety & Schmidt, 1948). The integrative relationship between PaCO₂ and pH on CBF regulation 75 acts to stabilize CO₂ gradients and thus regulate pH across the blood-brain-barrier; that is, 76 alterations in PaCO₂ provoke inverse changes in pH (Fencl *et al.*, 1969; reviewed in: Hoiland *et* 77 al., 2019). Although acute changes in respiratory acidosis/alkalosis elicit changes in 78 interstitial/intracellular pH (Fencl et al., 1966; Betz & Heuser, 1967; Arieff et al., 1976), the CSF 79 pH is stable across chronic metabolic acidosis and alkalosis to support a narrow range of 80 extravascular pH levels irrespective of marked changes in arterial pH (Mitchell et al., 1965; 81 Fencl et al., 1969; reviewed in: Siesjö, 1972). It is noteworthy that PaCO₂-mediated 82 cerebrovascular responses are dependent on the rapid diffusion of CO₂ across the vascular wall 83 to alter perivascular extracellular pH rather than direct changes in arterial pH per se (Wolff & 84 Lennox, 1930; Lambertsen et al., 1961; Severinghaus & Lassen, 1967; Betz & Heuser, 1967; 85 Wahl et al., 1970; Kontos et al., 1977b; 1977a). With this view, at least in the context of acute 86 metabolic alkalosis, CBF regulation is dependent on PaCO₂ rather than arterial pH per se 87 (Caldwell *et al.*, 2021); however, whether this finding is consistent following acclimatization to 88 high-altitude, where metabolic compensation for the prevailing respiratory alkalosis occurs, 89 merits investigation. 90 Alterations in acid-base balance during high-altitude exposure are well-reported

91 (Dempsey et al., 1974; Forster et al., 1975; Weiskopf et al., 1976). With ascent to high-altitude,

92 the initial hypoxic ventilatory response reduces $PaCO_2$ and arterial [H⁺] (i.e., respiratory

93 alkalosis) and, as such, arterial/CSF pH is elevated (Severinghaus et al., 1963; reviewed in:

94 Hoiland *et al.*, 2018). This respiratory alkalosis is partially compensated by renal excretion of

95 bicarbonate (HCO₃⁻) that begins in the first 1-2 days across progressive acclimatization; e.g., 1-2

96 weeks (Gledhill et al., 1975; Dempsey et al., 1978; Krapf et al., 1991). Acetazolamide (ACZ) is

97 a carbonic anhydrase inhibitor that accelerates the acclimatization process (Bärtsch & Swenson,

- 98 2013; Swenson, 2014) by increasing ventilation and accelerated renal excretion of HCO₃⁻ to
- 99 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 PCO₂ (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

107 vasoconstriction (Willie et al., 2012; 2015).

108 According to the Henderson-Hasselbalch equation: $pH = 6.1 + \log [HCO_3] / (0.0314 \times$ 109 PCO₂) (Hasselbalch, 1916), reductions in arterial [HCO₃⁻] at high-altitude would decrease 110 buffering capacity; that is, a given change in $PaCO_2$ will elicit a larger change in arterial $[H^+]/pH$ 111 (Siesjö, 1972). As such, appropriate changes in cerebrovascular CO₂ reactivity (i.e., change in 112 CBF for a given change in $PaCO_2$) with respect to changes in buffering capacity are essential to 113 tightly regulate cerebral interstitial pH to support critical enzymatic function (Fencl et al., 1966; 114 reviewed in: Fencl & Rossing, 1989). Likewise, if the cerebral vasculature is acutely regulated 115 by PaCO₂ per se (Schieve & Wilson, 1953; Lambertsen et al., 1961; Caldwell et al., 2021), then 116 alterations in the buffering capacity of $PaCO_2$ and $[H^+]/pH$ at high-altitude will result in 117 consistent changes with CBF and $[H^+]/pH$ (i.e., rightward x-intercept shifts). Previous reports 118 indicate either attenuated (Ainslie et al., 2008), unchanged (Ainslie & Burgess, 2008; Rupp et 119 al., 2014; Willie et al., 2015), or augmented (Fan et al., 2010; Lucas et al., 2011; Flück et al., 120 2015) cerebrovascular CO₂ reactivity with initial ascent and partial acclimatization to high-121 altitude. These disparate findings are likely attributable to differences in approaches to index 122 cerebrovascular CO₂ reactivity, severity of altitude, ascent profile, stage of acclimatization, and 123 the prevailing compensatory changes in the buffering capacity of $PaCO_2$ and $[H^+]/pH$ at high-124 altitude (Crawford & Severinghaus, 1978; Mathew et al., 1983; Fan et al., 2015). 125 This study investigated the interaction between acid-base balance on resting CBF and 126 cerebrovascular CO_2 reactivity via three separate experimental trials at sea level and following 127 partial acclimatization (14 to 20 days) at 5,050 m, involving: 1) resting acid-base balance 128 (control); 2) following two days of oral acetazolamide dosing (ACZ; 250 mg per 8 hours); and 3) 129 after acute normalization of arterial pH (ACZ+HCO₃⁻). To account for the relative metabolic 130 acidosis elicited by ACZ, we utilized an intravenous infusion of sodium bicarbonate (NaHCO $_3^{-}$)

131 to normalize arterial pH with a partial restoration of PaCO₂. At least at sea level, intravenous 132 $NaHCO_3$ infusion provokes progressive increases in PaCO₂ via respiratory suppression (Gesell 133 et al., 1930; Shock & Hastings, 1935; Bernthal, 1937; Hesser, 1949; Singer et al., 1956) to 134 partially compensate for the associated metabolic alkalosis; however, this elevated arterial pH is 135 only reflected in the CSF/extracellular pH after several hours (Robin et al., 1958; Bradley & 136 Semple, 1962; Hornbein & Pavlin, 1975; Nattie & Romer, 1978; Abeysekara et al., 2012). 137 Whether these compensatory changes in respiration occur at high-altitude and their resultant 138 influence on cerebrovascular regulation has not been reported. We hypothesized that CBF 139 regulation at rest would correspond to changes in PaCO₂ (e.g., respiratory compensation) rather 140 than arterial pH (e.g., pharmacologically induced) between trials at sea level and high-altitude. 141 Additionally, we hypothesized that the cerebrovascular responses to changes in [H⁺]/pH would 142 be consistent with the altered relationship between $PaCO_2$ and $[H^+]/pH$ between altitudes and 143 within the acute acid-base trials. 144 145 **METHODS** 146 Ethical Approval 147

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.

153

154 Participants

155 Eleven healthy adults (n = 9 males/ 2 females; 28 ± 6 years, 175 ± 6 cm, 77 ± 14 kg, 25 ± 4

156 kg/m²) participated in this study at sea level (SL). Ten healthy adults (n = 7 males/ 3 females; 29

157 \pm 5 years, 175 \pm 6 cm, 74 \pm 12 kg, 24 \pm 4 kg/m²) participated in this study at high-altitude (HA).

- 158 Participants had no history of cardiovascular, cerebrovascular, or respiratory disease and were
- 159 not taking any cardiovascular medications.
- 160
- 161 <u>Experimental Overview</u>

162 This study was part of a larger research expedition conducted in April-June 2012. As such, 163 participants were involved in a number of studies conducted during pre-testing in Kelowna and 164 during the 3 weeks at the Ev-K2-CNR Pyramid Laboratory. The recovery time between the 165 various testing sessions was managed to avoid any cross-over effects between multiple 166 experiments (e.g., >48 hours between all drug and/or exercise intervention studies). The 167 experimental questions addressed in this study were a priori driven; however, a subset of 168 participants' resting arterial blood gas variables have been reported elsewhere in a separate 169 report (at sea level only) on the influence of ACZ on the pulmonary vascular pressure response 170 to acute hypoxia and blood flow through intrapulmonary arteriovenous anastomoses (Tremblay 171 et al., 2015).

172

173 Ascent to High-Altitude

174 All variables and measurements were obtained at the University of British Columbia Okanagan

175 Campus in Kelowna, BC, Canada (SL: 344 m, barometric pressure (Pb) 732 ± 16 mmHg) and

176 following 14 to 20 days at the Ev-K2-CNR Pyramid Laboratory, Khumbu Valley, Nepal (HA:

177 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

179 Bazaar, 3,440 m; Pengboche, 3,995 m; Pheriche, 4,371 m). Additionally, during the first 6-7

180 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).

182 Treatment of acetazolamide was discontinued on day 8 of the trek at 4,371 m to allow sufficient

183 time (e.g., >24 hours) for the drug to clear participants' system before the control trial at 5,050 m

184 (Ritschel *et al.*, 1998; Richalet *et al.*, 2005). This approach was utilized to provide a safe ascent

185 of the experimental volunteers at 5,050 m.

186

187 Protocol 1

188 At SL and following 14 to 20 days at HA participants first completed a control visit including a

189 standardized intra-cranial cerebrovascular CO₂ reactivity (CVR) test including stepwise iso-oxic

- alterations in PaCO₂ (hypo- and hypercapnia) in the following order: -10, -5, +0, +5, +10, +15
- 191 mmHg $PaCO_2$ via dynamic end-tidal forcing. The alterations in $PaCO_2$ were calculated from the
- 192 resting eupneic breathing end-tidal values obtained prior to each of the three experimental trials

- 193 at each altitude. Participants were unable to tolerate $+15 \text{ mmHg PaCO}_2$ at 5,050 m; therefore, the
- 194 hypercapnic CVR range was completed up to $+10 \text{ mmHg PaCO}_2$ at HA. Each stage of the
- 195 cerebrovascular CO₂ reactivity protocol lasted approximately 3 minutes to allow a steady-state
- 196 responsiveness to be achieved (Carr *et al.*, unpublished). All cardiorespiratory, cerebrovascular,
- 197 and arterial blood gas variables presented were measured within the last minute of each stage,
- 198 representative of a steady-state.
- 199

200 Protocol 2 & 3

- 201 Following the control visit, participants were prescribed an oral dose of ACZ (250 mg) every 8
- 202 hours for 2 days before their next visit. The last dose of ACZ was taken 1 hour before

203 experimentation. The cerebrovascular CO₂ reactivity protocol was then repeated twice (separated

- by at least 30 minutes) without intravenous NaHCO₃⁻ (ACZ) and with intravenous NaHCO₃⁻
- 205 (ACZ+HCO₃⁻). To allow for experimental alteration of arterial pH from a setting of relative
- 206 metabolic acidosis caused by ACZ, the 8.4% intravenous NaHCO₃⁻ solution (Hospira, Montreal,
- 207 Quebec, Canada) was delivered over a 15-minute infusion to acutely restore arterial pH to resting208 levels.
- 209

210 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 (20gauge; 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

- 216 sampling system (Edwards Lifesciences, TruWave VAMP, CA, USA) for repeated
- 217 measurements. All blood gas samples were analyzed immediately using a calibrated blood gas
- 218 analyzer (ABL90 FLEX, Radiometer). This analysis included measurements of the partial
- 219 pressures of arterial carbon dioxide (PaCO₂) and oxygen (PaO₂), arterial oxygen saturation
- 220 (SaO₂), bicarbonate ion concentration ($[HCO_3^-]$), hydrogen ion concentration ($[H^+]$), hemoglobin
- 221 concentration ([Hb]), hematocrit (HCT) and arterial pH. All samples were heated/corrected to an
- assumed resting body temperature of 37.0°C.

223

- Blood gas analyzers do not typically have the capacity to directly measure ([HCO₃⁻]); instead, it
- is calculated from measured PaCO₂ and pH, using the Henderson-Hasselbalch equation
- 226 (Hasselbalch, 1916). The pKa (i.e., -log of the acid dissociation constant) at 37.0°C of 6.1
- 227 (Cullen *et al.*, 1925) and the solubility factor for dissolved CO₂ plus carbonic acid (H₂CO₃) at
- 228 37.0°C in plasma of 0.0314 mmol·l⁻¹ per mmHg PaCO₂ were used:
- 229

230 $pH = 6.1 + \log [HCO_3^-] / (0.0314 \times PaCO_2)$

231

At SL, arterial blood samples were obtained from the radial artery prior to each cerebrovascular

- 233 CO₂ reactivity trial (e.g., control, ACZ, ACZ+HCO₃⁻) in a subgroup of participants (n=7) and
- again in five participants following the ACZ+HCO₃⁻ protocol to confirm that arterial pH and
- [HCO₃] 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 CO₂
- reactivity protocols for the control and ACZ trials; the ACZ+HCO₃⁻ trial was conducted on the
- next day at HA and arterial blood samples were obtained in a subgroup of participants (n=5).
 239
- At both SL and HA the deficit in $[HCO_3^-]$ was calculated from resting arterial $[HCO_3^-]$ taken
- 241 with and without ACZ and using body mass to calculate the required dosage of NaHCO₃⁻ with
- the below equations (Kollef & Isakow, 2012).
- 243
- Apparent volume of distribution = total body weight (kg) \times (0.4 + (2.4 / ACZ [HCO₃]))
- 245 Target change in $[HCO_3^-] = resting [HCO_3^-] ACZ [HCO_3^-]$

246 mEq of NaHCO₃⁻ = Apparent volume of distribution × target change in [HCO₃⁻] × 0.5

- 247
- 248 Arterial blood samples were obtained following NaHCO₃⁻ infusion to confirm sufficient
- normalization to control values. In the event that [HCO₃] was not completely restored to resting
- 250 levels additional NaHCO₃⁻ was administered and arterial [HCO₃⁻] levels were reassessed before
- 251 experimentation to confirm adequate restoration. The order of experiments was not randomized
- 252 because of the lasting effects of ACZ and $NaHCO_3^{-}$.
- 253
- 254 Cardiorespiratory

255 Breath-by-breath CO₂ and O₂ were sampled at the mouth and recorded using a gas analyzer 256 calibrated prior to each experimental session (ML206, ADInstruments, CO, USA). The partial 257 pressures of end-tidal CO₂ and O₂ (i.e., P_{ET}CO₂ and P_{ET}O₂, respectively) were calculated in 258 LabChart (ADInstruments) using peak detection analysis with correction for daily barometric 259 pressure at BTPS. Both P_{ET}CO₂ and P_{ET}O₂ were controlled using a custom-designed dynamic 260 end-tidal forcing system to effectively regulate end-tidal gases across wide ranges of P_{ET}CO₂ and 261 $P_{ET}O_2$ independent of ventilation (\dot{V}_E); this device has previously been described in detail 262 elsewhere (Tymko et al., 2015; 2016). Notably, this method of arterial blood gas alteration 263 attenuates the end-tidal-to-arterial PCO₂ gradient, precludes any influence of \dot{V}_E on 264 cerebrovascular CO₂ reactivity (Howe et al., 2020), and therefore provides an accurate stimulus-265 response relationship (Fisher, 2016; Fisher et al., 2018). Respiratory flow, tidal volume (V_T), and 266 respiratory frequency (f_R) were measured by a pneumotachograph (HR 800 L, Hans Rudolph, 267 Shawnee, KS, USA). Instantaneous minute ventilation (\dot{V}_E in liters per minute) was determined 268 as the product of breath-by-breath inspired volume (V_T; calculated from the integral of the flow 269 signal) and respiratory frequency ($f_{\rm R}$, in breaths per minutes; calculated by 60/period of the flow 270 signal).

271

272 Cardiovascular

273 At HA, beat-by-beat arterial blood pressure was acquired via the radial artery pressure transducer 274 positioned at the height of the right atrium (Edwards Lifesciences, TruWave VAMP, CA, USA). 275 At SL, continuous non-invasive blood pressure was acquired using finger photoplethysmography 276 (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands) and was calibrated prior 277 to data collection using the return-to-flow function and normalized to manual brachial artery 278 blood pressure measurements. The arterial and finger photoplethysmography blood pressure 279 waveforms were averaged to calculate MAP at each altitude, respectively. Heart rate was 280 continuously measured using a lead-II electrocardiogram (ECG; ML132 BioAmp, 281 ADInstruments, CO, USA). Peripheral oxygen saturation (SpO_2) was measured continuously by

282 pulse oximetry (ML320/F; ADInstruments, CO, USA).

283

284 Cerebrovascular

285 At rest, extra-cranial blood velocity and vessel diameter of the left internal carotid artery (ICA) 286 and right vertebral artery (VA) were measured using a 10-MHz multifrequency linear array 287 Duplex ultrasound (Terason t3000; Teratech, Burlington, MA, USA). Pulse-wave mode was 288 used to measure peak blood velocity and arterial diameter was instantaneously measured using 289 B-mode imaging. The ICA blood velocity and vessel diameter were measured ≥ 1.5 cm from the 290 carotid bifurcation to avoid any turbulent or retrograde flow patterns, while VA blood velocity 291 and diameter were measured between C4-C5 or C5-C6. The vessel location was decided on an 292 individual basis to allow for reliable image acquisition, with the same location and consistent 293 insonation angle (60°) repeated within participants and between trials. Our between-day 294 coefficients of variation for Q_{ICA} and Q_{VA} are 5% and 11%, respectively (Willie *et al.*, 2012). 295 Intra-cranial cerebral blood velocity (CBV) was assessed at rest and during CO₂ reactivity via 296 transcranial Doppler (TCD) ultrasound (Spencer Technologies, Seattle, WA, USA), as an index 297 of CBF, in the left middle cerebral artery (MCA) and right posterior cerebral artery (PCA). The 298 2-MHz TCD probes were attached to a specialized headband (model M600 bilateral head frame, 299 Spencer Technologies), and each vessel was insonated through the trans-temporal window, using 300 previously described location and standardization techniques (Willie et al., 2011). Our between-301 day coefficients of variation for MCAv and PCAv are 3% and 2%, respectively (Smith et al., 302 2012).

303

304 Data Analyses

305 Cardiovascular and respiratory measures were sampled continuously at 1000 Hz using an 306 analogue-to-digital converter (Powerlab/16SP ML795; ADInstruments, Colorado Springs, CO, 307 USA) and data were interfaced with LabChart (Version 7.1) and analyzed offline. Cardiovascular 308 and respiratory variables presented are 1-minute averages during steady-state conditions after ≥ 2 309 minutes at each stage of the CO₂ reactivity protocol. The Q_{ICA} and Q_{VA} recordings were at least 310 1-minute for each measurement (Thomas et al., 2015). Duplex ultrasound recordings were screen 311 captured and saved for offline analysis using custom edge-detection and wall tracking software 312 (BloodFlow Analysis, version 5.1). This analysis method utilizes integration of diameter and 313 velocity traces to calculate mean beat-to-beat flow at 30 Hz independent of observer bias 314 (Woodman et al., 2001). 315

316	Blood flow was calculated as:
317	$Q (\text{mL} \cdot \text{min}^{-1}) = \text{peak envelope blood velocity} / 2 \times (\pi (0.5 \times \text{diameter})^2) \times 60.$
318	
319	Global cerebral blood flow (gCBF) was calculated as:
320	$g\text{CBF}(\text{mL}\cdot\text{min}^{-1}) = 2 \times (Q_{\text{ICA}} + Q_{\text{VA}})$
321	
322	Arterial oxygen content (CaO ₂) was calculated as:
323	$CaO_2 (mL \cdot dL^{-1}) = [Hb] \times 1.34 \times [SaO_2 (\%)/100] + 0.003 \times PaO_2$
324	
325	Where 1.34 is the O_2 binding capacity of hemoglobin and 0.003 is the solubility of O_2 dissolved
326	in blood (Lumb, 2016; West & Luks, 2020).
327	
328	Cerebral oxygen delivery (CDO ₂) was calculated as:
329	$CDO_2 (mL \cdot min^{-1}) = gCBF \times CaO_2$
330	MCA or PCA DO ₂ (au) = MCAv or PCAv × CaO ₂
331	
332	Cerebrovascular conductance (CVC) was calculated as:
333	CVC (mL·min ⁻¹ ·mmHg ⁻¹) = gCBF, Q_{ICA} , Q_{VA} , MCA ν , or PCA ν / MAP
334	
335	<u>Statistical Analyses</u>
336	All data are presented as mean \pm SD. Statistical analyses were performed using SPSS software
337	(IBM statistics, Version 23.0) and statistical significance was set at P≤0.05. Comparisons were
338	made between SL and HA at rest between trials (control, ACZ, $ACZ+HCO_3$), and between
339	PaCO ₂ stages within elevation. A linear mixed-model analysis with compound symmetry
340	covariance structure with fixed effects of trial (control, ACZ, $ACZ+HCO_3$) and altitude (SL vs.
341	HA) was used to compare arterial blood gas, cardiorespiratory, and cerebrovascular variables at
342	rest. Resting MAP, PaCO ₂ , pH, [HCO ₃ ⁻], and CaO ₂ were added as covariates alongside trial and
343	altitude as fixed effects and subjects as a random effect for resting g CBF. The selected variables
344	were chosen as they are considered important regulators of CBF in humans (Willie et al., 2014)
345	and they each improved the model fit (-2 Log Likelihood), indicating their acceptability in the
346	model. A Bonferroni correction was applied for multiple comparisons when significant

- 347 interactions were detected. A linear mixed-model analysis with fixed effects of trial (control,
- 348 ACZ, ACZ+HCO₃) and stage (PaCO₂ level) was used during the cerebrovascular CO₂ reactivity
- 349 protocol for separate SL and HA comparisons. Separate hypo- and hypercapnic CVR was
- analyzed using linear regression to calculate the individual slope response between
- 351 cerebrovascular parameters and P_{ET}CO₂. A one-tailed, paired Student's t-test was used to
- 352 compare the individual x-intercept values for the absolute PaCO₂ versus pH and MCAv versus
- 353 pH cerebrovascular CO₂ reactivity slopes between each experimental trial at HA.
- 354
- 355 RESULTS
- 356
- 357 <u>Arterial blood gases</u>
- 358 Between altitudes within trials at rest: As expected, HA resulted in arterial hypoxemia (PaO₂: Δ -
- 359 53 ± 6 mmHg and SaO₂: Δ -13.4 \pm 1.3 %, altitude effects: P < 0.001 and P < 0.001, respectively;
- **Table 1**) and respiratory alkalosis (PaCO₂: Δ -14.7 ± 2.3 mmHg and pH: Δ +0.03 ± 0.04, altitude
- 361 effects: P < 0.001 and P = 0.002, respectively; **Table 1**) with partial metabolic compensation
- 362 ([HCO₃⁻]: Δ -7.6 ± 1.4 mEq·l⁻¹, within trials all P < 0.001; **Table 1**). Overall, CaO₂ was lower at
- HA versus SL during control and ACZ trials (P < 0.001 and P < 0.001, respectively; **Table 1**)
- 364 with no change between altitudes during the ACZ+HCO₃⁻ trial (P = 0.433; **Table 1**).
- 365
- 366 *Between trials across altitudes at rest:* Across altitudes, arterial pH was lower following ACZ
- 367 versus control ($\Delta -0.07 \pm 0.04$, trial effect: P < 0.001; **Table 1**) and ACZ+HCO₃⁻ ($\Delta -0.06 \pm 0.04$,
- trial effect: P < 0.001; **Table 1**); that is, ACZ+HCO₃⁻ effectively restored arterial pH to control
- 369 values at both SL and HA. Following ACZ, arterial [HCO₃⁻] was lower at SL (Δ -6.3 ± 2.0
- 370 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,
- 371 NaHCO₃⁻ infusion effectively normalized arterial [HCO₃⁻] to control values at each altitude (P =
- 372 0.279 and P = 0.060, respectively; **Table 1**). Following ACZ at SL and HA, PaCO₂ was lower (Δ
- -3.4 ± 2.3 mmHg, trial effect: P < 0.001; **Table 1**) and PaO₂ was higher ($\Delta + 6.3 \pm 6.5$ mmHg,
- trial effect: P = 0.003; **Table 1**) with no change between control and ACZ+HCO₃⁻ trials (trial
- effect: P = 0.053 and P = 0.458, respectively; **Table 1**). At SL, CaO₂ was higher following ACZ
- 376 versus control ($\Delta + 1.2 \pm 1.0 \text{ mL} \cdot \text{dL}^{-1}$, P < 0.001; **Table 1**) and ACZ+HCO₃⁻ ($\Delta + 1.2 \pm 1.0$

377 mL·dL⁻¹, P = 0.001; **Table 1**) trials. At HA, CaO₂ was also higher following ACZ ($\Delta + 1.8 \pm 1.1$

378 mL·dL⁻¹, P < 0.001; **Table 1**) and ACZ+HCO₃⁻ (Δ +1.7 ± 1.4 mL·dL⁻¹, P < 0.001; **Table 1**) 379 versus the control trial.

380

381 <u>Cardiorespiratory</u>

382 There was no interaction between the resting \dot{V}_E , HR, and MAP responses between control,

383 ACZ, and ACZ+HCO₃⁻ trials and SL versus HA (all P > 0.05; **Table 2**). Throughout the CO₂

reactivity tests, there was no interaction between the \dot{V}_E , HR, and MAP responses between trials

at either altitude (all P > 0.05). Across altitudes, MAP was lower at rest and throughout the CO_2

reactivity tests during the ACZ trial compared to control and ACZ+HCO₃⁻ trials (trial effects: all

- 387 P < 0.05; **Table 2**).
- 388

389 <u>Cerebrovascular</u>

- 390 There was no interaction between the resting CDO_2 , gCBF, and $gCBF_{CVC}$ responses between
- 391 control, ACZ, and ACZ+HCO₃⁻ trials and SL versus HA (all P > 0.05; **Table 2**). Resting CDO₂,
- 392 *g*CBF, and *g*CBF_{CVC} were all higher at HA versus SL (altitude effects: all P < 0.05; **Table 2**).
- Further, covariate analysis revealed no significant influence of resting $PaCO_2$, pH, or $[HCO_3^-]$ on
- resting CDO₂, gCBF, and gCBF_{CVC} responses between trials and altitudes (all P > 0.05). As
- such, there was no correlation between the absolute resting PaCO₂, [H⁺], or pH and the

396 respective gCBF 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+HCO₃⁻ experimental

401 trials. As ACZ provokes reductions in both arterial pH and PaCO₂ (**Table 1**) – via metabolic

- 402 acidosis and elevated respiration it is important to consider the directional alterations in gCBF
- 403 responses with these respective competing changes in $PaCO_2$ and $[H^+]/pH$ between trials at SL
- 404 and HA. Overall, an unchanged gCBF response corresponded with a higher arterial $[H^+]$ (i.e.,
- 405 lower pH) as well as a reduction in PaCO₂ following ACZ; that is, respiratory compensation (i.e.,
- 406 Δ PaCO₂), rather than the prevailing arterial [H⁺]/pH, is sufficient to offset any changes in gCBF
- 407 with ACZ and ACZ+HCO₃⁻ acid-base alterations at SL and HA.

408

409 *High-altitude MCAv CO₂ reactivity regulation:* Throughout the CO₂ reactivity tests at HA. 410 arterial pH was lower during the ACZ trial than control (Δ -0.08 ± 0.01, trial effect: P < 0.001; 411 **Figure 2**) and ACZ+HCO₃⁻ (Δ -0.03 ± 0.02, trial effect: P < 0.001; **Figure 2**). The arterial pH 412 was lower during the ACZ+HCO₃⁻ versus control trial CO₂ reactivity test (Δ -0.05 ± 0.02, trial effect: P < 0.001; Figure 2) even though arterial pH was not different at rest between 413 414 ACZ+HCO₃⁻ and control trials (P = 0.060). Across PaCO₂ stages, CaO₂ was higher than the control trial with both ACZ ($\Delta + 1.4 \pm 0.4 \text{ mL} \cdot \text{dL}^{-1}$, trial effect: P < 0.001) and ACZ+HCO₃⁻¹ (Δ 415 $+1.7 \pm 0.5 \text{ mL} \cdot \text{dL}^{-1}$, trial effect: P < 0.001); as such, MCA DO₂ was not different between trials 416 417 (trial effect: P = 0.622). Across the full range of PaCO₂ alterations, there was no difference 418 between the sensitivity (i.e., slope) of the absolute MCAy response to changes in PaCO₂, $[H^+]$, or 419 pH (P = 0.156, P = 0.238, and P = 0.073, respectively) across trials at HA (Figure 2 A & B). 420 Additionally, absolute MCAv was not different at each stage of $PaCO_2$ between trials at HA (P = 421 0.913).

422

423 Hypocapnic versus hypercapnic reactivity: Within altitudes at SL and HA, there was no 424 difference between either the absolute MCAv or PCAv (each covariate adjusted by MAP; all P < 425 0.001) versus P_{ET}CO₂/PaCO₂ responses, respectively across the full range of CO₂ reactivity (i.e., 426 inclusive of hypo- and hypercapnia) between control, ACZ, and ACZ+HCO₃ (all P > 0.05; 427 Figure 3A & 3B). Across trials, the separate hypocaphic and hypercaphic relative CVR slopes 428 were higher at HA versus SL for MCAv, PCAv, MCA_{CVC}, and PCA_{CVC} (altitude effects: all P < 429 0.05; **Table 3 & Figure 3C**). Across altitudes, absolute and relative MCAv hypercapnic CVR 430 were both higher during the control trial than ACZ and ACZ+HCO₃⁻ with no influence of 431 altitude *per se* (trial effects: P = 0.004 and P = 0.005; **Tables 3-4 & Figure 3D**). At HA, relative 432 PCA_{CVC} hypocapnic CVR was lower during the ACZ+HCO₃⁻ trial than control (-15 \pm 24%, P = 433 0.028) and ACZ (-21 \pm 18%, P = 0.003). At SL, the absolute hypocaphic CVR was higher during the ACZ+HCO₃⁻ trial than control for MCAv ($+0.8 \pm 0.7 \text{ cm} \cdot \text{s}^{-1}$ per mmHg, P = 0.003), MCA_{CVC} 434 $(+0.01 \pm 0.01 \text{ cm} \cdot \text{s}^{-1} \text{ per mmHg}, P = 0.004)$, and PCA_{CVC} $(+0.01 \pm 0.00 \text{ cm} \cdot \text{s}^{-1} \text{ per mmHg}, P = 0.004)$ 435 0.021) (Table 4). These between trial differences in the absolute hypocaphic CVR responses to 436 437 $ACZ+HCO_3^{-}$ were not apparent at HA. 438

439 DISCUSSION

440

441 The results of this study indicate that in the context of acute and chronic changes in arterial pH 442 the CBF response is consistent with changes in PaCO₂ rather than the prevailing arterial $[H^+]/pH$ 443 *per se.* These data support the view that the buffering capacity of extracellular rather than 444 intravascular arterial $[H^+]/pH$ gradients regulate CBF as cerebrovascular CO₂ reactivity was 445 consistently higher following partial acclimatization to high-altitude versus sea level irrespective 446 of experimentally controlled metabolic acidosis/alkalosis. This finding is supported by: 1) resting 447 CBF and cerebrovascular CO₂ reactivity were unchanged between trials within each altitude 448 even though arterial pH and $[HCO_3^-]$ (i.e., buffering capacity) were effectively altered; and 2) 449 intra-individual responses at rest indicate reductions in PaCO₂ (via respiratory compensation) are 450 sufficient to regulate gCBF with metabolic acidosis rather than the countervailing changes in 451 arterial [H⁺]/pH. Taken together, within the experimental constraints of this study, these findings 452 indicate that CO₂-mediated changes in cerebrovascular regulation rather than *arterial* $[H^+]/pH$ is 453 integral in the regulation of CBF in humans following acclimatization to high-altitude.

454

455 *Cerebrovascular regulation following acute and chronic alterations in acid base balance:*

456 Resting CBF was unaltered between trials within each altitude even though arterial pH and 457 [HCO₃] (i.e., buffering capacity) were effectively reduced and restored with ACZ and 458 $ACZ+HCO_3$ trials, respectively (**Table 1**). These results are inconsistent with previous reports at 459 HA of approximately 25% increases in resting CBF following 2 hours of a large oral ACZ 460 ingestion (1.5 g; 3,475 m) (Jensen et al., 1990) or rapid 60-s intravenous infusion (10 mg/kg; 461 5,050 m) (Fan et al., 2012). It is noteworthy, however, that these studies were performed acutely 462 with a higher relative ACZ dose than the current experiment (2 days; 250 mg per 8 hours) and 463 such doses/intravenous approaches are not ecologically valid at HA (Low et al., 2012; Bärtsch & 464 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 465 466 altering cerebral metabolism (Posner & Plum, 1960; Vorstrup et al., 1984); importantly, such 467 acute intravenous protocols do not alter $PaCO_2$ whereas the expected hyperventilatory response 468 was observed in the present study following 2 days oral ACZ (e.g., Δ -5 mmHg PaCO₂; **Table** 469 1). Further, reports indicate ACZ infusion attenuates the regulatory rise in CSF $[HCO_3]$ in

- 470 response to increases in PaCO₂ (Wichser & Kazemi, 1975; Kazemi *et al.*, 1976; Shibata *et al.*,
- 471 1976; Kazemi & Choma, 1977); therefore, *intravenous* ACZ may indeed exacerbate reductions
- 472 in buffering capacity, necessitating an increase in CBF to maintain extravascular pH (Skinhoj,
- 473 1966; Severinghaus & Lassen, 1967; Lassen, 1968; Fencl *et al.*, 1969).
- 474 Well-established *in vivo* pre-clinical studies show that changes in PaCO₂ rather than 475 arterial pH per se mediate alterations in CBF by stabilizing perivascular pH (Betz & Heuser, 476 1967; Wahl et al., 1970; Betz et al., 1973; Kontos et al., 1977b; 1977a). As such, a rightward 477 shift in the PaCO₂-pH relationship at altitude and resultant reduction in buffering capacity (via 478 lower [HCO₃⁻]) would also result in a rightward shift in the CBF-pH relationship. This view is 479 consistent with recent results from Caldwell and colleagues (2021) that show a rightward x-480 intercept shift in both the PaCO₂-pH and CBF-pH responses following acute metabolic alkalosis 481 via intravenous NaHCO₃⁻ at SL. Relatedly, between trials at HA, we observed *leftward* shifts in 482 both the PaCO₂-pH and CBF-pH relationships with experimentally *reduced* arterial pH (e.g., 483 ACZ; Figure 2A & 2C). These data indicate that changes in the x-intercept of CBF versus pH 484 were consistent with the altered relationship between PaCO₂ and pH at HA; i.e., the sensitivity of 485 CBF to $PaCO_2$ per se was unchanged between trials at HA. At least at SL, previous studies 486 report that metabolic acidosis has an equivalent or additive influence on the hypoxic ventilatory 487 response (Forster & Klausen, 1973; Swenson & Hughes, 1993); however, the absolute change in 488 PaCO₂ with ACZ was approximately 60% less at HA than at SL (Table 1). As discussed next, 489 the leftward shift in the CBV-P_{ET}CO₂ response with ACZ at SL can likely be attributed to the 490 larger absolute change in PaCO₂ with ACZ at SL (Figure 3A).
- 491

492 *Cerebrovascular regulation is not exclusively regulated by arterial pH:* These data support

- 493 recent pre-clinical work which substantiates that CO₂ signalling via astrocytic CO₂/HCO₃⁻
- 494 transport mediates CBF regulation (e.g., neurovascular coupling) independently of
- 495 experimentally altered arterial/extracellular pH (Hosford *et al.*, 2021; *preprint*). Additionally,
- 496 these results are consistent with evidence that PCO₂-mediated release of ATP (via CO₂-sensitive
- 497 connexin-26 proteins) independent of extracellular acidosis and Ca^{2+} is integral to the ventilatory
- 498 response to CO₂ (Huckstepp *et al.*, 2010; Cummins *et al.*, 2020); however, whether this CO₂
- 499 signalling is involved with CBF regulation requires investigation. The ACZ-induced reductions
- 500 in arterial pH would theoretically increase gCBF if metabolic acidosis is considered exclusively

501 (Fencl et al., 1969). Rather, gCBF was statistically unaltered between experimental trials within 502 each altitude, indicating that respiratory compensation (i.e., reductions in $PaCO_2$) was sufficient 503 to offset any elevations in gCBF expectedly induced by ACZ-mediated metabolic acidosis. In 504 support of this finding, the intra-individual responses indicate that the reductions in PaCO₂ are 505 consistent with the directional alterations in gCBF with ACZ and ACZ+HCO₃⁻ trials (Figure 1). 506 Following ACZ at SL, resting PaCO₂ was reduced by approximately 5 mmHg (**Table 1**); taken 507 together with the well-established hypocapnic CVR of approximately 3-4% per mmHg PaCO₂ 508 (Kety & Schmidt, 1948; Ramsay et al., 1993; Ito et al., 2000; 2003; Willie et al., 2012; 509 Coverdale et al., 2014; reviewed in: Hoiland et al., 2019), these data indicate that the extent of 510 respiratory compensation to ACZ is apparently exaggerated with respect to the unaltered gCBF. 511 In support of this view, previous reports show a progressive 30% reduction in alveolar PCO₂ 512 following 10 days of oral ACZ (500 mg twice daily); alongside this respiratory compensation, total CBF (via intravenous xenon¹³³ technique) was restored to control values within 2 days of 513 514 ACZ treatment and throughout the following 15 days indicating a countervailing influence of 515 acid-base balance on cerebrovascular regulation (Lassen et al., 1987; Friberg et al., 1990).

516

517 The relationship between arterial PCO₂ and H^+/pH predicates cerebrovascular CO₂ reactivity at 518 high-altitude: The present results are consistent with other studies that have reported higher 519 cerebrovascular CO₂ reactivity with initial ascent and partial acclimatization to HA (Fan *et al.*, 520 2010; 2012; 2014; Flück et al., 2015). Within each altitude, there were selective alterations in 521 absolute and relative hypocaphic CVR during the ACZ+HCO₃⁻ trial (**Tables 3-4**); such findings 522 are perhaps attributable to relative increases in buffering capacity via exogenously elevated 523 arterial [HCO₃⁻] (Siesjö, 1972) and/or direct effects of extracellular [HCO₃⁻] on cerebrovascular tone via changes in vascular smooth muscle cell contractility and Ca^{2+} sensitivity (Boedtkjer *et* 524 525 al., 2016; Boedtkjer, 2018). As the NaHCO₃⁻ infusion acutely restored pH – rather than 526 promoting further metabolic alkalosis – it is likely that these effects are due to the exogenous 527 increases in arterial [HCO₃] rather than pH per se. It is noteworthy that the absolute MCA_V was 528 not different at each stage of PaCO₂ between experimental trials at HA (Figure 2B). Pioneering 529 work by Severinghaus and colleagues (1963) revealed tight regulation of CSF pH with chronic 530 hypocapnia and arterial alkalosis following 8 days of acclimatization to 3,800 m – although the 531 capacity of CSF [HCO₃⁻] active transport is reportedly controversial, these data illustrate the

532 importance of interstitial/extracellular pH regulation (Severinghaus et al., 1963; Severinghaus,

533 1965; Mitchell et al., 1965; Pappenheimer1970, 1970; Hasan & Kazemi, 1976; Kazemi &

- 534 Choma, 1977; Bledsoe *et al.*, 1981). Overall, the hyperventilatory-induced reductions in PaCO₂
- 535 (i.e., respiratory alkalosis) correspond with the slow exchange of CSF $[HCO_3^-]$ to normalize CSF
- 536 pH and CBF with progressive acclimatization, further substantiating that arterial pH *per se* does
- 537 not dictate CBF regulation. We interpret the unaltered CBF-PaCO₂ response between trials at
- 538 HA to indicate that CBF is acutely regulated by PaCO₂ within the context of acute and chronic
- 539 alterations in arterial pH following partial acclimatization to 5,050 m.
- 540

541 Experimental considerations: With this experimental protocol we were restricted by the lasting 542 effects of ACZ and NaHCO₃⁻ on acid-base balance; therefore, the order of trials were not 543 randomized within altitudes. Without an appropriate time-control, changes in CaO₂ may have 544 occurred due to acclimatization to HA throughout the testing sessions that lasted two days 545 following 14-20 days at 5,050 m; however, this is unlikely as we observed a related increase in 546 CaO₂ 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 547 548 direct vasodilatory influence of ACZ on the systemic circulation (Parati et al., 2013; Eskandari et al., 2018) via opening of Ca²⁺-activated K⁺ channels (Pickkers et al., 2001). Notwithstanding, 549 550 covariate analysis revealed no influence of resting MAP on gCBF within each trial and the 551 resting gCBF_{CVC} response was unaffected by ACZ at both altitudes (**Table 2**). Previous reports 552 indicate that cerebral oxidative metabolism is higher at rest following 4-6 days at 5,050 m (Smith 553 et al., 2014), unaltered following 5 weeks at 5,260 m (Møller et al., 2002), and likely varies with 554 acute alterations in PaCO₂ (Willie *et al.*, 2015). As such, future investigations on the relationship 555 between cerebrovascular acid-base regulation and metabolism at high-altitude are merited. It is 556 unknown whether ACZ may differentially affect cerebral oxidative metabolism at high-altitude. 557

558 Technical considerations: A strength of the current study was the use of regional volumetric

- 559 $Q_{\rm ICA}$ and $Q_{\rm VA}$ to calculate gCBF at rest; however, such an approach would be preferable to
- 560 MCAv/PCAv estimates of CBF throughout the cerebrovascular CO₂ reactivity tests. Duplex
- 561 ultrasound facilitates B-mode arterial diameter in the sagittal axis and pulse-wave blood velocity
- 562 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 MCAv systematically underestimates Duplex ultrasound and Fick-derived CBF reactivity to PaCO₂ 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.

569 Additionally, continuous arterial blood gas sampling throughout the CO₂ reactivity tests 570 at SL would have been advantageous to directly ascertain the changes in buffering capacity with 571 respiratory acidosis/alkalosis. Bland-Altman analysis revealed that the resting PETCO2-PaCO2 572 gradients at SL and HA were 1.0 ± 3.1 and 3.7 ± 1.6 mmHg, respectively; consistent with 573 previously reported values (Willie et al., 2012; Tymko et al., 2015). Importantly, the linearly related $P_{ET}CO_2$ -PaCO₂ gradient (R² 0.94, P < 0.001) throughout the cerebrovascular CO₂ 574 575 reactivity tests at HA was consistent with the gradient observed at rest (e.g., 3.7 ± 1.6 vs. $3.7 \pm$ 576 1.7 mmHg); therefore, the small (<3 mmHg) P_{ET}CO₂-PaCO₂ gradient at HA versus SL likely did 577 not alter our findings. Relatedly, the validity of arterial pH and acid-base buffering as an index of 578 CSF pH changes at HA deserves consideration. Reductions in CSF [HCO₃⁻] follow changes in 579 arterial blood indicating that passive exchange of CO₂ across the blood-brain-barrier and 580 resultant re-equilibrium of the reaction between CO₂ and HCO₃⁻ provokes changes in CSF 581 [HCO₃] and pH (Forster et al., 1975; Weiskopf et al., 1976). Dempsey and colleagues (1974) 582 reported consistent CSF to arterial pH gradients (Δ -0.08 pH units) and closely matched [HCO₃⁻] 583 between CSF and arterial samples at SL and following 3-4 weeks at 3,100 m. Additionally, they 584 reported that the relative partial metabolic/respiratory compensation with acclimatization to HA 585 was not different between arterial blood and CSF with respect to $[H^+]/pH$ and PCO₂ changes; as 586 such, these data support the efficacy of arterial acid-base changes as an index of CSF regulation. 587

588 CONCLUSION

589

590 These findings reveal that in the context of acute and chronic changes in arterial pH – via partial

591 acclimatization to high-altitude and experimentally controlled metabolic acidosis/alkalosis –

592 including within trial acute alterations in PaCO₂ (i.e., respiratory acidosis/alkalosis), the CBF

response is consistent with changes in PaCO₂ rather than the prevailing arterial $[H^+]/pH$ per se.

594	In support of this, we show that resting CBF and the cerebrovascular reactivity to $PaCO_2$ were
595	unchanged between trials within each altitude even though arterial pH and [HCO ₃ ⁻] (i.e.,
596	buffering capacity) were effectively altered. Taken together, these findings are consistent with
597	previous studies indicating PaCO ₂ and resultant passive diffusion of CO ₂ across the vascular wall
598	to alter perivascular pH, rather than arterial pH per se, acutely regulates CBF in humans.
599	
600	Data Availability Statement
601	The data that support the findings of this study are available from the corresponding author upon
602	reasonable request.
603	
604	Competing Interests
605	None to declare.
606	
607	Author Contributions
608	PNA, SJEL, MS, and KRB conceived and designed the research. KJS, NL, RLH, CKW, SJEL,
609	MS, KRB, DBM, and PNA acquired the data. HGC and RLH analyzed the data. HGC, RLH, and
610	PNA interpreted the data. HGC drafted the manuscript. All authors revised the manuscript and
611	provided intellectual feedback and agree to be accountable for all aspects of the work.
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617	
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624	of this research study.

625

626 TABLE CAPTIONS

627

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)

630

631 Abbreviations: Hydrogen concentration, $[H^+]$; bicarbonate concentration, $[HCO_3^-]$; arterial

- $carbon dioxide tension, PaCO_2$; arterial oxygen tension, PaO₂; arterial oxygen saturation, SaO₂;
- arterial oxygen content, CaO_2 ; hemoglobin concentration, [Hb]. Trial main effect pairwise comparisons: ^aP < 0.05 versus control; ^bP < 0.05 versus ACZ. Trial × altitude interaction
- 634 comparisons: ${}^{a}P < 0.05$ versus control; ${}^{b}P < 0.05$ versus ACZ. Trial × altitude interaction 635 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; $^{\&}P$
- 637 < 0.05 versus ACZ+HCO₃ between altitudes. Data are mean \pm SD. Sample sizes: n=7 for all
- 638 three trials at sea level (SL), n=10 for the control and ACZ trials at high-altitude (HA), and n=5
- 639 for the ACZ+ HCO_3^- trial at HA.
- 640

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)

643

644 Abbreviations: Ventilation, \dot{V}_E ; tidal volume, V_T ; respiratory frequency, f_R ; breaths per minute,

- 645 BPM; heart rate, HR; beats per minute, bpm; mean arterial pressure, MAP; cerebral oxygen
- 646 delivery, CDO₂; global cerebral blood flow, *g*CBF; global cerebrovascular conductance,
- 647 $gCBF_{CVC}$. Trial main effect pairwise comparisons: ^bP < 0.05 versus ACZ. Trial × altitude
- 648 interaction pairwise comparisons: ${}^{6}P < 0.05$ versus ACZ between altitudes. Data are mean \pm SD
- 649 for n=11 at sea level (SL) and n=10 at high-altitude (HA).
- 650

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)

653

654 Abbreviations: Middle cerebral artery blood velocity, MCA*v*; Posterior cerebral artery blood

- 655 velocity, PCA*v*; cerebrovascular conductance, CVC; hypocapnia, hypo-; hypercapnia, hyper-.
- 656 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; $^{\&}P$
- 659 < 0.05 versus ACZ+HCO₃ between altitudes, 1 < 0.05 versus ACZ+Control, 659 < 0.05 versus ACZ+HCO₃ between altitudes. Data are mean \pm SD. Sample sizes: SL control,
- n=10; SL ACZ, n=11; SL ACZ+HCO₃⁻, n=11; HA control, n=10; HA ACZ, n=9; HA
- 661 ACZ+HCO₃, n=9.
- 662
- 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)
- 664 665
- 666 Abbreviations: Middle cerebral artery blood velocity, MCAv; Posterior cerebral artery blood
- 667 velocity, PCAv; cerebrovascular conductance, CVC; hypocapnia, hypo-; hypercapnia, hyper-.
- 668 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+HCO₃⁻ between

altitudes. Data are mean \pm SD. Sample sizes: SL control, n=10; SL ACZ, n=11; SL ACZ+HCO₃,

672 n=11; HA control, n=10; HA ACZ, n=9; HA ACZ+HCO₃, n=9.

673

674 FIGURE CAPTIONS

675

676 Figure 1. Global cerebral blood flow (gCBF) regulation at rest during acetazolamide (ACZ) and 677 acetazolamide with bicarbonate (ACZ+HCO₃) trials at sea level (A & C) and high-altitude (B & 678 **D**). Each panel shows the intra-individual variability between the absolute change in arterial H^+ 679 (A & B) and PCO₂ (PaCO₂) (C & D) and the respective relative change in gCBF. The absolute 680 change in H⁺ and PaCO₂, and the relative change in gCBF are compared to the control trial 681 values, respectively. Overall, an unchanged gCBF response corresponded with a higher arterial 682 $[H^+]$ (i.e., lower pH) as well as a reduction in PaCO₂ following ACZ; that is, respiratory 683 compensation (i.e., $\Delta PaCO_2$), rather than the prevailing arterial [H⁺]/pH, is sufficient to offset 684 any changes in gCBF with ACZ and ACZ+HCO₃⁻ acid-base alterations at SL and HA. Data are 685 individual values with group averages. Sample sizes: n=8 for SL both trials, n=8 for ACZ at HA,

- 686 and n=5 for ACZ+HCO₃⁻ at HA.
- 687
- 688 Figure 2. Acid-base balance and cerebrovascular regulation throughout CO₂ reactivity tests
- during control, acetazolamide (ACZ) and acetazolamide with bicarbonate (ACZ+HCO₃) trials at
- 690 high-altitude. There was a significant leftward shift in the x-intercept in the absolute MCAv
- 691 versus pH response within the ACZ versus control trial (7.59 \pm 0.04 vs. 7.71 \pm 0.08, P = 0.002);
- 692 however, this was reversed with ACZ+HCO₃ (7.65 \pm 0.04 vs. 7.71 \pm 0.08, P = 0.086) (A). These
- 693 leftward x-intercept shifts were consistent with the PaCO₂ versus pH response between trials
- (C); i.e., the altered relationship between PaCO₂-pH was reflected in a leftward shift in the
- 695 MCAv-pH response. It is noteworthy that the absolute MCAv was not different at each stage of
- $PaCO_2$ between trials at HA (**B**). Throughout the CO₂ reactivity tests, CaO₂ was not significantly
- 697 different between trials when indexed against arterial pH (**D**). Data are mean \pm SD for n=10 for 698 control & ACZ and n=5 for ACZ+HCO₃⁻.
- 699
- Figure 3. Cerebrovascular regulation throughout CO₂ reactivity tests during control,
- acetazolamide (ACZ) and acetazolamide with bicarbonate (ACZ+HCO₃⁻) trials at sea level (SL)
- and high-altitude (HA). At SL, the MCA_{CVC}-P_{ET}CO₂ response was leftward shifted with ACZ
- and ACZ+HCO₃⁻ and this was likely explained by the significant reduction in *resting* PaCO₂
- within these trials (A). As the absolute change in resting $PaCO_2$ with ACZ and ACZ+HCO₃⁻ was
- less at HA, there was no difference between the MCA_{CVC} -PaCO₂ responses between trials (**B**).
- 706 The MCAv hypo- and hypercapnic CVR was consistently higher at HA compared to SL
- irrespective of trial (C & D). Across altitudes, MCAv hypercapnic CVR was higher during the
- control trial than ACZ and ACZ+HCO₃ with no influence of altitude *per se* (**D**). Data are mean
- \pm SD (**A** & **B**) and individual values with group averages (**C** & **D**). Sample sizes: (**A**) n=10 for
- 710 control and n=11 for ACZ & ACZ+HCO₃; (**B**) n=10 for control & ACZ and n=5 for
- 711 ACZ+HCO₃; (C) & (D) n=10 for SL control, n=11 for SL ACZ & ACZ+HCO₃, n=10 for HA
- 712 control, n=9 for HA ACZ & ACZ+HCO $_3$.

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		Control	ACZ	ACZ+HCO3		P-Values	
					Trial	Altitude	Trial imes Altitude
лU	SL	7.42 ± 0.01^{b}	7.36 ± 0.03	7.44 ± 0.02^{b}	P < 0.001	P = 0.002	P = 0.117
pm	HA	7.47 ± 0.07^{b}	7.40 ± 0.02	7.44 ± 0.02^{b}		1 - 0.002	
$[\mathbf{H}^{+}](\mathbf{n}\mathbf{M})$	SL	$37.9 \pm 1.3^{\rm b}$	44.0 ± 3.2	36.6 ± 2.1^{b}	P < 0.001	D = 0.005	P = 0.172
	HA	34.4 ± 6.8^{b}	39.8 ± 2.1	36.0 ± 1.6^{b}		r = 0.005	
$[HCO_3^-]$	SL	$25.5 \pm 1.6^{\$,+}$	$19.2 \pm 0.9^{a,\%}$	$24.6 \pm 1.2^{a,b,\$,\&}$	D < 0.001	D < 0.001	D = 0.002
$(mEq \cdot l^{-1})$	HA	$17.4 \pm 2.5^{\$,+}$	$13.5 \pm 1.3^{a,\%}$	$15.9 \pm 1.2^{a,b,\$,\&}$	P < 0.001	P < 0.001	P = 0.002
Base Excess	SL	$1.3 \pm 1.4^{\mathrm{b},\mathrm{S},\mathrm{+}}$	$-5.4\pm0.8^{\%}$	$0.7 \pm 1.2^{b,\$,\&}$	D . 0.001	D . 0.001	D 0.025
$(mEq \cdot l^{-1})$	HA	$-6.3 \pm 3.6^{b,\$,+}$	$-11.3 \pm 1.4^{\%}$	$-8.2 \pm 1.2^{b,\$,\&}$	P < 0.001	P < 0.001	P = 0.025
$D_{0}CO_{1}(mmH_{0})$	SL	40.1 ± 3.6	35.2 ± 3.5^{a}	37.1 ± 2.9	P < 0.001	P < 0.001	P = 0.073
$FacO_2$ (mmrg)	HA	23.7 ± 1.8	21.7 ± 2.1^{a}	23.2 ± 2.2			
$D_{2}O_{1}$ (mm U_{2})	SL	94 ± 13	102 ± 7^{a}	98 ± 9	P = 0.005	D < 0.001	P = 0.703
PaO_2 (IIIIIIHg)	HA	43 ± 2	48 ± 2^{a}	46 ± 3		P < 0.001	P = 0.705
$\mathbf{S}_{\mathbf{n}}\mathbf{O}_{\mathbf{n}}(0(1))$	SL	$97.0\pm1.6^{+}$	$97.8 \pm 0.5^{ m a,\%}$	$97.5 \pm 0.7^{ m a,\&}$	D < 0.001		D 0.029
$SaO_2(\%)$	HA	$82.7\pm2.2^+$	$85.0 \pm 2.2^{a,\#,\%}$	$85.0 \pm 2.4^{\mathrm{a}, \#, \&}$	P < 0.001	P < 0.001	P = 0.038
	SL	$19.8 \pm 0.7^{\$,+}$	$21.0 \pm 0.5^{a,\%}$	$19.7 \pm 1.0^{\mathrm{a,b,\$}}$	D 0 001	D 0.001	D 0.002
CaO_2 (mL·dL)	HA	$17.5\pm1.1^+$	$19.3 \pm 1.2^{a,\#,\%}$	$19.1 \pm 0.5^{a,b,\#}$	P < 0.001	P < 0.001	P = 0.003
	SL	$15.0 \pm 0.7^{\$,+}$	$15.8 \pm 0.4^{a,\%}$	$14.9 \pm 0.8^{a,b,\$,\&}$	P < 0.001	D . 0.001	D 0.024
[Hb] (g·al)	HA	$15.7\pm1.2^{\scriptscriptstyle +}$	$16.9 \pm 1.1^{a,\#,\%}$	$16.6 \pm 0.9^{a,b,\#,\&}$		P < 0.001	P < 0.001

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, PaC₂; arterial oxygen saturation, SaO₂; arterial oxygen content, CaO₂; hemoglobin concentration, [Hb]. Trial main effect pairwise comparisons: ^aP < 0.05 versus control; ^bP < 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+HCO₃⁻ 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+HCO₃⁻ trial at HA.

		Control	ACZ	ACZ+HCO3 ⁻		P-Values	
					Trial	Altitude	Trial imes Altitude
$\dot{\mathbf{V}}$ (I min ⁻¹)	SL	11.5 ± 2.2	11.8 ± 1.4	12.0 ± 1.3	P = 0.434	D < 0.001	D = 0.912
$v_{\rm E}$ (L·mm)	HA	17.6 ± 3.7	19.1 ± 1.5	18.7 ± 3.6		r < 0.001	F = 0.813
V (I)	SL	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	P = 0.499	D < 0.001	P = 0.085
$\mathbf{v}_{\mathrm{T}}(\mathrm{L})$	HA	1.2 ± 0.4	1.1 ± 0.1	1.3 ± 0.2		F < 0.001	r = 0.085
f (DDM)	SL	14 ± 3	$14 \pm 2^{\%}$	16 ± 2	D = 0.662	D = 0.026	P = 0.046
$J_{\rm R}$ (BPNI)	HA	16 ± 5	$18 \pm 2^{\%}$	15 ± 2	P = 0.663	P = 0.020	
UD (hnm)	SL	59 ± 6	60 ± 9	60 ± 10	P = 0.126	P < 0.001	P = 0.066
пк (орш)	HA	72 ± 12	76 ± 14	70 ± 14			
MAD(mmHa)	SL	$86 \pm 8^{\mathrm{b}}$	82 ± 9	$85 \pm 11^{\mathrm{b}}$	P = 0.004	D < 0.001	P = 0.083
MAP (mmig)	HA	$105 \pm 7^{\mathrm{b}}$	92 ± 7	102 ± 7^{b}		F < 0.001	
CDO ₂	SL	88 ± 43	89 ± 31	91 ± 42	D = 0.679	D = 0.000	D = 0.627
$(mL \cdot min^{-1})$	HA	120 ± 43	125 ± 38	108 ± 13	$\Gamma = 0.078$	P = 0.009	F = 0.027
gCBF	SL	450 ± 204	423 ± 152	460 ± 204	D 0.256	D < 0.001	D 0 205
$(mL \cdot min^{-1})$	HA	683 ± 225	647 ± 189	607 ± 120	P = 0.330	P < 0.001	P = 0.295
gCBF _{CVC}	SL	5.38 ± 2.25	5.11 ± 1.77	5.45 ± 2.28			
(mL·min ⁻	~ -				P = 0.583 $P = 0.022$	P = 0.022	P = 0.218
¹ ·mmHg ⁻¹)	HA	6.54 ± 2.02	7.15 ± 1.88	5.92 ± 0.99			

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, CDO₂; global cerebral blood flow, *g*CBF; global cerebrovascular conductance, *g*CBF_{CVC}. Trial main effect pairwise comparisons: ^bP < 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).

		Control	ACZ	ACZ+HCO3		P-Values	
Reactivity (Δ % per Δ mmHg)					Trial	Altitude	Trial × Altitude
MCAv hypo-	SL HA	$\begin{array}{c} 3.0\pm0.5\\ 4.2\pm0.8\end{array}$	$\begin{array}{c} 2.9\pm0.4\\ 4.0\pm0.5\end{array}$	$\begin{array}{c} 3.3\pm0.5\\ 4.2\pm0.5\end{array}$	P = 0.276	P < 0.001	P = 0.413
MCAv hyper-	SL HA	4.7 ± 1.1 7.1 ± 1.6	$\begin{array}{c} 3.7 \pm 1.1^{a} \\ 6.0 \pm 1.0^{a} \end{array}$	$\begin{array}{c} 3.2 \pm 1.0^{a} \\ 6.4 \pm 1.5^{a} \end{array}$	P = 0.005	P < 0.001	P = 0.424
MCA _{CVC} hypo-	SL HA	$\begin{array}{c} 2.9 \pm 0.5^{+} \\ 3.7 \pm 0.7^{+} \end{array}$	$\begin{array}{c} 2.8 \pm 0.3 ^{\%} \\ 3.9 \pm 0.4 ^{\%} \end{array}$	$\begin{array}{c} 3.2\pm0.6\\ 3.6\pm0.6\end{array}$	P = 0.539	P < 0.001	P = 0.045
MCA _{CVC} hyper-	SL HA	2.8 ± 1.0 3.1 ± 1.2	$\begin{array}{c} 2.5\pm0.7\\ 2.6\pm0.7\end{array}$	$\begin{array}{c} 2.1 \pm 0.7^{\&} \\ 3.4 \pm 1.0^{\&} \end{array}$	P = 0.252	P = 0.018	P = 0.030
PCAv hypo-	SL HA	$\begin{array}{c} 2.7 \pm 0.3^{+} \\ 4.4 \pm 0.8^{+} \end{array}$	$\begin{array}{c} 3.0 \pm 0.5^{\%} \\ 4.2 \pm 0.7^{\%} \end{array}$	$\begin{array}{c} 3.2 \pm 0.8^{\&} \\ 3.9 \pm 0.5^{\&} \end{array}$	P = 0.805	P < 0.001	P = 0.006
PCAv hyper-	SL HA	4.1 ± 1.2 7.9 ± 2.2	$\begin{array}{c} 3.9\pm1.5\\ 6.7\pm0.8\end{array}$	$3.3 \pm 1.2 \\ 6.6 \pm 2.2$	P = 0.073	P < 0.001	P = 0.470
PCA _{CVC} hypo-	SL HA	$\begin{array}{c} 2.6 \pm 0.3^{+} \\ 3.9 \pm 0.7^{+} \end{array}$	$\begin{array}{c} 2.9 \pm 0.5 ^{\%} \\ 4.1 \pm 0.5 ^{\%} \end{array}$	$\begin{array}{c} 3.1 \pm 0.8 \\ 3.2 \pm 0.7^{\#,\$} \end{array}$	P = 0.081	P < 0.001	P = 0.001
PCA _{CVC} hyper-	SL HA	2.4 ± 1.1 3.7 ± 1.6	2.7 ± 1.2 3.1 ± 0.6	2.2 ± 0.9 3.4 ± 1.4	P = 0.849	P = 0.002	P = 0.418

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, MCAv; Posterior cerebral artery blood velocity, PCAv; 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+HCO₃⁻ between altitudes. Data are mean ± SD. Sample sizes: SL control, n=10; SL ACZ, n=11; SL ACZ+HCO₃⁻, n=11; HA control, n=10; HA ACZ, n=9; HA ACZ+HCO₃⁻, n=9.

		Control	ACZ	ACZ+HCO3		P-Values	
Reactivity (Δ cm/s per Δ mmHg)					Trial	Altitude	Trial × Altitude
MCAv hypo-	SL HA	$\begin{array}{c} 2.0 \pm 0.4^+ \\ 2.8 \pm 0.8^+ \end{array}$	$\begin{array}{c} 2.1\pm0.4\\ 2.6\pm0.7\end{array}$	$\begin{array}{c} 2.7 \pm 0.7^{\#,\$} \\ 2.7 \pm 0.6 \end{array}$	P = 0.073	P = 0.028	P = 0.016
MCAv hyper-	SL HA	3.1 ± 0.7 4.7 ± 1.1	$\begin{array}{c} 2.6\pm0.8^a\\ 3.8\pm0.7^a\end{array}$	$\begin{array}{c} 2.5\pm0.6^{a}\\ 3.6\pm1.1^{a} \end{array}$	P = 0.004	P < 0.001	P = 0.699
MCA _{CVC} hypo-	SL HA	$\begin{array}{c} 0.02 \pm 0.00 \\ 0.02 \pm 0.01 \end{array}$	$\begin{array}{c} 0.03 \pm 0.01 \\ 0.03 \pm 0.01 \end{array}$	$\begin{array}{c} 0.03 \pm 0.01^{\#,\&} \\ 0.02 \pm 0.01^{\&} \end{array}$	P = 0.081	P = 0.127	P = 0.012
MCA _{CVC} hyper-	SL HA	$\begin{array}{c} 0.02 \pm 0.01 \\ 0.02 \pm 0.01 \end{array}$	$\begin{array}{c} 0.02 \pm 0.01 \\ 0.02 \pm 0.00 \end{array}$	$\begin{array}{c} 0.02 \pm 0.01 \\ 0.02 \pm 0.01 \end{array}$	P = 0.498	P = 0.037	P = 0.911
PCAv hypo-	SL HA	1.3 ± 0.5 2.0 ± 0.5	1.4 ± 0.4 1.8 ± 0.5	1.7 ± 0.7 1.8 ± 0.5	P = 0.447	P = 0.017	P = 0.056
PCAv hyper-	SL HA	2.0 ± 0.8 3.6 ± 1.1	$\begin{array}{c} 1.8\pm0.7\\ 2.9\pm0.8\end{array}$	1.7 ± 0.6 2.9 ± 0.7	P = 0.084	P < 0.001	P = 0.427
PCA _{CVC} hypo-	SL HA	$\begin{array}{c} 0.01 \pm 0.00 \\ 0.02 \pm 0.00 \end{array}$	$\begin{array}{c} 0.02 \pm 0.00 \\ 0.02 \pm 0.01 \end{array}$	$\begin{array}{c} 0.02 \pm 0.01^{\#,\&} \\ 0.01 \pm 0.00^{\&} \end{array}$	P = 0.168	P = 0.385	P = 0.015
PCA _{CVC} hyper-	SL HA	$\begin{array}{c} 0.01 \pm 0.01 \\ 0.02 \pm 0.01 \end{array}$	$\begin{array}{c} 0.01 \pm 0.01 \\ 0.01 \pm 0.01 \end{array}$	$\begin{array}{c} 0.01 \pm 0.01 \\ 0.01 \pm 0.00 \end{array}$	P = 0.940	P = 0.199	P = 0.577

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; ${}^{s}P < 0.05$ versus ACZ within altitude; ${}^{+}P < 0.05$ versus control between altitudes; ${}^{e}P < 0.05$ versus ACZ+HCO₃⁻ between altitudes. Data are mean ± SD. Sample sizes: SL control, n=10; SL ACZ, n=11; SL ACZ+HCO₃⁻, n=11; HA control, n=10; HA ACZ, n=9; HA ACZ+HCO₃⁻, n=9.











 Δ MCAv (%) / Δ $P_{ET}CO_2$ (mmHg)