Integrated respiratory chemoreflex-mediated regulation of cerebral blood flow in hypoxia: implications for oxygen delivery and acute mountain sickness

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ABSTRACT

The aim of the present study was to determine to what extent hypoxia-induced changes in the peripheral and central respiratory chemoreflex modulate anterior and posterior cerebral blood flow (CBF), oxygen delivery (CDO_2) and corresponding implications for the pathophysiology of the neurological syndrome, acute mountain sickness (AMS). Eight healthy males were randomly assigned single-blind to 7 h passive exposure to both normoxia (21% O_2) and hypoxia (12% O_2). The peripheral and central respiratory chemoreflex, internal carotid (ICA), external carotid (ECA), and vertebral (VA) artery blood flow (duplex ultrasound) and AMS scores (questionnaires) were measured throughout. A reduction in ICA CDO_2 was observed during hypoxia despite a compensatory elevation in perfusion. In contrast, VA and ECA CDO_2 were preserved that in the former was due to a more marked increase in perfusion. Hypoxia was associated with progressive activation of the peripheral respiratory chemoreflex (P <0.001) whereas the central respiratory chemoreflex remained unchanged (P>0.05). Symptom severity in participants who developed clinical AMS was positively related to ECA blood flow (LLS, r = 0.546~0.709, P = 0.004~0.043; ESQ-C, r = 0.587~0.771, P = 0.001~0.027, n=4). Collectively, these findings highlight the site-specific regulation of CBF in hypoxia that selectively maintains CDO_2 in the posterior but not anterior cerebral circulation, with minimal contribution from the central respiratory chemoreflex. Furthermore, ECA vasodilation may represent a hitherto unexplored hemodynamic risk factor implicated in the pathophysiology of AMS.

New Findings

What is the central question of this study?

To what extent does hypoxia-induced changes in the peripheral and central respiratory chemoreflex modulate anterior and posterior cerebral oxygen delivery and corresponding implications for acute mountain sickness susceptibility?

What is the main finding and its importance?

Evidence for site-specific regulation of cerebral blood flow in hypoxia that preserves oxygen delivery in the posterior but not anterior cerebral circulation, with minimal contribution from the central respiratory chemoreflex. External carotid artery

61 vasodilation may prove an alternative hemodynamic risk factor that predisposes to
62 acute mountain sickness.
63
64 **Key Words:** Hypoxia; cerebral blood flow; oxygen delivery; respiratory chemoreflex,
65 acute mountain sickness

Introduction

The human brain has evolved exquisite sensitivity to oxygen (O_2)/glucose supply and carbon dioxide (CO_2) clearance given its disproportionately high mass-specific energy demands, limited energy stores and almost exclusive reliance on aerobic metabolism (Bailey, 2019b, a). To protect against potential damage caused by hypoxia, the brain is equipped with specialized defence mechanisms including the carotid (peripheral) and medullary (central) respiratory chemoreflexes, capable of 'sensing' subtle changes in blood O_2/CO_2/H^+ concentrations and relaying signals that ultimately preserve bioenergetic homeostasis (Costa *et al.*, 2014; Dempsey *et al.*, 2014).

While hypoxia is a potent cerebral vasodilator reflected by an elevation in CBF in direct proportion to the severity of isocapnic hypoxemia, activation of the peripheral respiratory chemoreflex in poikilocapnia leads to hyperventilation-induced hypocapnia and subsequent cerebral vasoconstriction, antagonizing CDO_2 (Ainslie *et al.*, 2016; Hoiland *et al.*, 2016). Furthermore, hypoxic regulation of CBF and CDO_2 is heterogenous with emergent, albeit controversial evidence for preferential vasodilatation in the posterior (brainstem) as opposed to the anterior (cortical) circulation reflected by increased flow through the vertebral (VA) relative to the internal carotid (ICA) arteries (Willie *et al.*, 2012; Ogoh *et al.*, 2013b). Furthermore, there is evidence to suggest that the posterior circulation exhibits comparatively lower sympathetic innervation (Edvinsson *et al.*, 1976), dynamic cerebral autoregulation (Sato *et al.*, 2012a), and CO_2 vasoreactivity (Sato *et al.*, 2012b) and is thus better equipped to conserve substrate delivery to phylogenetically older more important areas of the brain implicated in cardiorespiratory control.

Exposing adult humans to hypoxia alters pulmonary ventilation (Mahamed & Duffin, 2001), due in part to changes in the central chemoreflex (Sato *et al.*, 1992), peripheral chemoreflex (Robbins, 1995; Garcia *et al.*, 2001), and peripheral chemoreceptor-mediated depression of central respiratory drive (Dahan *et al.*, 1994; Kimura *et al.*, 1998). Indeed, (Mahamed & Duffin, 2001) demonstrated that repeated hypoxia exposure (20 min hypoxia once daily for 14 consecutive days) decreases the chemoreflex threshold and consequently enhances the ventilatory response to hypoxia. The central and peripheral respiratory chemoreflex that directly and indirectly affect

CBF regulation (Ogoh *et al.*, 2008; Ogoh *et al.*, 2009; Ogoh *et al.*, 2013a) may be modified during continuous hypoxia exposure, yet no studies to our knowledge that have defined the temporal kinetics and functional interaction between hypoxia-induced activation of the (peripheral/central) respiratory chemoreflex and corresponding implications for the differential modulation of CBF and CDO_2 across distinct vascular beds. Furthermore, while classically viewed as an evolutionary conserved adaptive trait, cerebral hypoxic vasodilatation may equally enhance vulnerability to AMS, a debilitating neurological syndrome characterized by headache and related vegetative sequelae (Bailey *et al.*, 2009a). Although the pathogenic significance of intracranial hypertension (IH) remains disputed, direct activation of the trigeminovascular system (TVS) subsequent to displacement of pain-sensitive unmyelinated fibres caused by IH is considered the primary stimulus underlying cephalalgia (Sanchez del Rio & Moskowitz, 1999). The TVS connects to intracranial and extracranial circulation (Lance, 1993), while it has been reported that intracranial CBF regulation did not affect AMS (Subudhi *et al.*, 2010). Yet to what extent extracranial vasodilatation that we have also linked to flow redistribution (vascular steal) from the ICA (Hirasawa *et al.*, 2016) and subsequent mechanical distention of terminal branches of the external carotid artery (ECA) that ultimately converge on the TVS potentially influence susceptibility to AMS remains to be investigated.

To address these knowledge gaps, we conducted a randomised, cross-over, single-blind study incorporating serial measurements in normoxia and hypoxia to test three functionally integrated hypotheses. First, that CDO_2 to the posterior circulation (VA) would remain well preserved (i.e. equivalent to normoxia) subsequent to a sustained elevation in CBF, whereas CBF and CDO_2 would become progressively lower in the anterior circulation ($\Delta VA > \Delta ICA$). Second, that differential reactivity would be related to selective activation of the peripheral respiratory chemoreflex and corresponding vasoconstrictive constraints imposed by hyperventilation-induced hypocapnia to which the anterior circulation is more sensitive combined with vascular steal from the ICA to the ECA. Finally, that the severity of AMS would be directly related to the hypoxia-induced increase in ECA vasodilatation given its anatomical proximity to the TVS.

Methods

Ethical Approval

The study was approved by the Ethics Committee of the University of South Wales, UK (#201712BS01). All procedures were carried out in accordance with the most (7th) recent amendment of the Declaration of Helsinki of the World Medical Association (with the exception that it was not registered in a publicly accessible database prior to recruitment) with verbal and written informed consent obtained from all participants.

Participants

Eight physically active male participants (age: 23 ± 2 y, stature: 1.81 ± 0.04 m, mass: 80 ± 7 kg) participated in this study. All participants lived close to sea level (90 m) and had not been exposed to simulated or terrestrial high-altitude in the previous 12 months. Following a medical examination, they were confirmed to be healthy and free of any known diseases. Furthermore, they were not taking any prescribed or over-the-counter medications or supplements. They were instructed to refrain from physical activity, caffeine, and alcohol and to follow a low nitrate/nitrite diet for at least 24 h prior to formal experimentation (Bailey *et al.*, 2017a).

Experimental Procedure

Following familiarization, participants completed two different experimental trials (randomised, cross-over, single-blind design, Fig. 1), in a normobaric environmental chamber (~120 m^3) maintained at 21°C and 50% relative humidity (Design Environmental, Ebbw Vale, UK). Participants were randomly assigned to complete 7 h passive exposure to normoxia (FiO_2 = 0.21) and 7 h of hypoxia (FiO_2 = 0.12), separated by 7 days. Participants arrived at the laboratory (between 8:00 and 9:00 A.M.) following a 12 h overnight fast and were fitted with an indwelling cephalic venous cannula. They consumed a standardized meal (30 g of oats with 180 mL water), 30 min prior to the experimental trials. They received the standardized meal again at 2 h, 4 h, and 6 h to maximize compliance and avoid hunger/dehydration (Bailey *et al.*, 2001). Throughout both exposures, blood samples, CBF (including the peripheral respiratory chemoreflex), cardiopulmonary variables, and AMS scores were assessed on the hour every hour for

the duration of exposure to each respective inspirate. The central respiratory chemoreflex was assessed at the 2, 4, and 6 h time-points.

Blood sampling

Whole blood was obtained at the various timepoints illustrated in Fig. 1 without stasis from an indwelling cannula located in a forearm antecubital vein. Haemoglobin (Hb) was measured photometrically in triplicate (average value taken) according to established procedures (Vanzetti, 1966) (HaemoCue®, B-Haemoglobin, Sheffield, UK).

Cardiopulmonary function

Heart rate (HR) was monitored by ECG (lead II) and SpO_2 was quantified via finger-pulse oximetry (WristOx2® 3150, Nonin, Minnesota, USA). Beat-to-beat arterial blood pressure (ABP) was monitored continuously using finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands). The finometer blood pressure waveform was used to indirectly measure ABP after calibrating values to the average of two automated brachial blood pressure measurements (Life Source, A&D Medical, model: UA767FAM), taken over a five-minute resting baseline period. Cardiac output ($\dot{Q}$) was determined from the ABP waveform using the BeatScope version 1.0 software (TNO-TPD, Biomedical Instrumentation, Amsterdam, The Netherlands), which incorporates the sex, age, stature, and mass of the participant. Respiratory rate (RR), tidal volume (V_T), minute ventilation ($\dot{V}_E$) and end-tidal partial pressures of O_2 and CO_2 ($P_{ET}O_2/P_{ET}CO_2$) were measured via a mouthpiece and automatic breath-by-breath respiratory gas-analyzing system that housed a differential pressure transducer, sampling tube, filter, suction pump and mass spectrometer (ML 206, ADInstruments, UK). Haemodynamics and respiratory measurements were recorded continuously at 1 kHz using an analog-to-digital converter (PowerLab; ADInstruments) and stored on a laboratory computer for offline analysis. These variables were averaged during the final 30 s of each hour.

Respiratory chemoreflex

Central chemoreflex: The sensitivity of the central respiratory chemoreflex was determined using an open-circuit apparatus at the 2, 4, and 6 h time-points. Participants breathed through a mouthpiece attached to a low-resistance one-way valve with a built-in hot-wire flow metre. The valve mechanism allowed participants to inspire room air or medical-grade gas mixture from a 200 L Douglas bag containing 5% CO_2 in 21% O_2 (normoxia) or 12% O_2 (hypoxia) with balanced nitrogen (N_2) (Ellingsen *et al.*, 1987a; Ellingsen *et al.*, 1987b). Increased $P_{ET}CO_2$ (input) stimulates chemoreceptor activity thereby increasing pulmonary ventilation ($\dot{V}_E$, output) via the central respiratory chemoreflex. The response of $\dot{V}_E$ to a change in (inspired) CO_2 is close to constant within the range of 0–5% CO_2 in the inspired gas (Ogoh *et al.*, 2008). To reduce participant stress, we employed two CO_2 inspirates (0.03% and 5% CO_2) to quantify the relative change in $\dot{V}_E$ in response to $P_{ET}CO_2$ ($\Delta\dot{V}_E$ / $\Delta P_{ET}CO_2$) as an index of central respiratory chemoreflex sensitivity.

Peripheral chemoreflex: We calculated the $\dot{V}_E$ response relative to changes in $P_{ET}O_2$ ($\Delta\dot{V}_E/\Delta P_{ET}O_2$) between the normoxic and hypoxic inspirates every hour given the constancy of each respective inspirate (i.e. we could not dynamically manipulate F_IO_2) given the potential confounds for all remaining variables of interest.

Cerebrovascular function

Volumetric blood flow in the ICA, ECA, and VA were determined by two separate investigators concurrently using duplex ultrasound (Vivid-i; GE Healthcare, Tokyo, Japan) equipped with a 10–13 MHz linear transducer. We and others have demonstrated that this approach is technically feasible and free of. any potential confounds caused by probe positioning or competitive ultrasound beam interference (Sato *et al.*, 2011; Ogoh *et al.*, 2014a; Hirasawa *et al.*, 2016; Ogoh *et al.*, 2017; Caldwell *et al.*, 2020). ICA and ECA measurements were taken 1.0–1.5 cm distal to the carotid bifurcation on the right side of the neck with the subject's chin slightly elevated. Left VA blood flow was measured between the transverse process of the C3 vertebra and subclavian artery. Specifically, the systolic and diastolic diameters were measured and used to calculate mean diameter in cm [(systolic diameter × 1/3) + (diastolic diameter × 2/3)]. The time-averaged mean

blood velocity (cm/s) was obtained using pulsed wave mode, averaged over 10–20 cardiac cycles to minimize potential effects caused by respiration (Sato *et al.*, 2011). When measuring blood velocity, care was taken to ensure that the probe position was stable, that the insonation angle was maintained throughout at 60 deg, the sample volume gate was positioned in the center of the vessel and adjusted to cover the width of the vessel.

Calculations

Total vascular conductance (TVC) was calculated by dividing $\dot{Q}$ by mean arterial pressure (MAP). Blood flow was calculated by multiplying the cross-sectional area [$\pi \times$ (mean diameter/2)^2] by mean blood flow; blood flow = mean blood velocity × area × 60 (mL/min). Global CBF (gCBF) was calculated as: (ICA blood flow + VA blood flow) × 2. Cerebral vascular conductance (CVC) at each artery was calculated by dividing CBF at each artery by MAP. In addition, we calculated the ICA, VA, and ECA CVC response relative to changes in $P_{ET}O_2$ or $P_{ET}CO_2$ between the normoxic and hypoxic inspirates every hour. The contributions of $\dot{Q}$ to ICA, ECA, and VA blood flows were calculated as: (ICA blood flow, ECA blood flow or VA blood flow) × 2/$\dot{Q}$ ×100 (%). Local and global CDO_2 were determined as CDO_2 (mL/min) = ICA blood flow, ECA blood flow, VA blood flow or gCBF × (estimated) arterial O_2 content (CaO_2), calculated as $\left(\text{Hb (g/dL)} \times 1.39 \times \frac{SaO_{2\,(\%)}}{100}\right)$ excluding (albeit minor) contributions from dissolved O_2 (0.003 x arterial PO_2) since we did not perform arterial catheterization.

Acute mountain sickness (AMS) scores

The Lake Louise Score (LLS) (Roach *et al.*, 2018) and Environmental Symptoms Questionnaires-cerebral symptoms score (ESQ-C) (Sampson *et al.*, 1983) were used to evaluate AMS symptoms every hour during both exposures. Participants were also asked to rate their cephalalgia using a clinically validated visual analog scale (0 to 100 mm; 0 mm = no headache, 10 mm = mild headache including a sensation of pressing or throbbing, 50 mm = moderate-intensity headache, and 100 mm = worst possible headache) (Iversen *et al.*, 1989). Clinical AMS (moderate-to-severe) was defined if a

participant presented with a LLS of ≥ 5 points in the presence of a headache and ESQ-C ≥ 0.7 points at the 6h time-point of exposure to hypoxia (Bailey *et al.*, 2006).

Statistical analysis

All data were analyzed using SPSS (IBM SPSS Statistics Version 25.0) and expressed as mean ± standard deviation (SD). Repeated Shapiro-Wilk W tests were employed to test for distribution normality. Data were analyzed using a combination of one and two (*Condition*: normoxia vs. hypoxia × *Time*: 1h-7h or *Artery*: ICA, VA vs. ECA × *Time*: 1h-7h) repeated measures analyses of variance (ANOVA). For non-normally distributed data, the Friedman test (nonparametric equivalent) was performed on ranks, and pairwise comparisons were made using Wilcoxon signed-ranks tests. Differences between means were located using Bonferroni-corrected paired samples *t*-tests when an interaction effect(s) was detected. Effect sizes were calculated retrospectively as *eta squared* (η^2) for all ANOVA outcomes according to established procedures (Lakens, 2013). Relationships between select dependent variables were determined using Spearman's rank-order correlations. Significance was determined at an alpha level of 0.05 for all two-tailed tests.

Results

Cardiopulmonary

MAP was lower whereas $\dot{Q}$ and TVC were elevated in hypoxia compared to normoxia (P = 0.039, η^2 = 0.166; P = 0.006, η^2 = 0.138 and P = 0.004, η^2 = 0.215, respectively) whereas HR did not change (P = 0.051, η^2 = 0.066, Table 1). As expected, $P_{ET}O_2$ and SpO_2 were lower during hypoxia (both, P < 0.001, η^2 > 0.825), but the decreased $P_{ET}O_2$ progressively increased toward the baseline throughout 7 h hypoxia (P = 0.001, η^2 = 0.296, Fig. 2). A progressive and equivalent elevation in Hb was observed normoxia and hypoxia (between trials P = 0.162, η^2 = 0.020). $P_{ET}CO_2$ was lower during hypoxia (P = 0.001, η^2 = 0.642), and gradually decreased throughout (P = 0.001, η^2 = 0.167). $\dot{V}_E$ was higher during hypoxia (P = 0.008, η^2 = 0.232) and this elevation in $\dot{V}_E$ was gradually increased throughout (P = 0.012, η^2 = 0.121). Central chemoreflex sensitivity ($\Delta\dot{V}_E$ / $\Delta P_{ET}CO_2$) did not change during hypoxia (P = 0.223, *Kendall's* W = 0.250, Fig. 3 A), whereas the peripheral chemoreflex sensitivity ($\Delta\dot{V}_E$ / $\Delta P_{ET}O_2$) progressively increased (P = 0.035, η^2 = 0.178, Fig3 B). No significant correlations were observed between these hemodynamic parameters and CBF CDO_2 (Table 2).

Cerebrovascular

ICA blood flow was generally elevated throughout hypoxia reaching its peak by the 2nd h before progressively decreasing over time (P = 0.024, η^2 = 0.182, Fig. 4 A). This was due primarily to a change in blood velocity that increased by the 2nd and 3rd h before decreasing thereon in (P = 0.012 vs. normoxia, Table 3), while diameter increased throughout hypoxia (P = 0.005, η^2 = 0.075). However, both elevations in ICA blood flow and ICA CVC (P = 0.002, η^2 = 0.243, Fig. 4B) were not sufficient to offset the hypoxia-induced reduction in CaO_2 (Table 1) resulting in lower ICA CDO_2 relative to normoxia (P = 0.009, η^2 = 0.186, Fig 4C). VA blood flow (P = 0.006, η^2 = 0.030) and VA CVC (P = 0.008, η^2 = 0.084) were consistently elevated during hypoxia and VA CDO_2 was comparable to that observed in normoxia (P = 0.414, η^2 =0.001). In contrast to ICA, VA diameter did not change (P = 0.195, η^2 = 0.007), whereas VA blood velocity tended to increase (P = 0.07, η^2 = 0.092). In contrast, gCBF CDO_2 was lower in hypoxia compared to normoxia (P = 0.019, η^2 = 0.080) despite an elevation in gCBF (P = 0.002, η^2 = 0.088) and gCVC (P = 0.002, η^2

296 = 0.209). Hypoxia failed to alter ECA blood flow and ECA CDO_2 (P = 0.212, η^2 = 0.048
297 and P = 0.181, η^2 = 0.051, respectively) but elevated ECA CVC (P = 0.021, η^2 = 0.192).
298 Hypoxia increased ECA diameter (P = 0.001, η^2 = 0.239) without affecting blood velocity
299 or flow (P = 0.813, η^2 = 0.002). ICA, VA and ECA CVC responses to $P_{ET}O_2$ and $P_{ET}CO_2$
300 remained unchanged throughout ($P_{ET}O_2$; P = 0.906, *Kendall's* W = 0.045, P = 0.667,
301 *Kendall's* W = 0.085 and P = 0.872, *Kendall's* W = 0.051, and $P_{ET}CO_2$; P = 0.448, *Kendall's*
302 W = 0.121, P = 0.546, *Kendall's* W = 0.104 and P = 0.448, *Kendall's* W = 0.121, Fig.5 A,
303 B). However, there was no difference in CVC to changes in $P_{ET}O_2$ and $P_{ET}CO_2$ between
304 arteries (P = 0.246, η^2 =0.040 or P = 0.670, η^2 =0.008).

305 The relative elevation in $\dot{Q}$ and TVC was augmented in hypoxia (P = 0.004, η^2
306 = 0.191 and P = 0.009, η^2 = 0.235, Fig.6 A, B) but the contribution of $\dot{Q}$ to total CBF
307 (sum of ICA, ECA and VA blood flow) decreased (P < 0.001, η^2 = 0.042, Fig.6 C). The
308 contribution of $\dot{Q}$ to VA and ECA blood flow did not change in hypoxia ($P \geq$ 0.331 and P
309 $\geq$ 0.180), whereas ICA blood flow decreased consistently throughout ($P \leq$ 0.024, Fig. 6 C).
310
311 ***AMS***
312 As expected, hypoxia increased AMS scores (Table 4) with four of eight participants
313 (50 %) diagnosed with clinical AMS and in whom ECA blood flow was selectively elevated
314 (P = 0.003 vs. non-AMS, η^2 = 0.260, Fig. 7). In addition, relationships were observed
315 between ECA blood flow and AMS symptom severity (Table 5).

Discussion

The present study has highlighted three important findings. First, ICA CDO_2 was consistently lower in hypoxia despite a compensatory elevation in ICA blood flow, whereas VA and ECA CDO_2 remained well preserved. Second, hypoxia was associated with progressive activation of the peripheral respiratory chemoreflex that likely attenuated the hypoxia-induced reduction in $P_{ET}O_2$ whereas the contribution from the central respiratory chemoreflex was minimal. Third, hypoxic vasodilatation of the ECA was consistently more pronounced in participants diagnosed with clinical AMS suggesting a potential link to underlying pathophysiology. Collectively, these findings highlight the integrated cerebrovascular response to the site-specific regulation of CDO_2 and corresponding neurological complications in hypoxia. That substrate delivery was better preserved in the posterior compared to the anterior cerebral circulation makes teleological sense given that the territories the vertebral-basilar system feeds, notably the medulla oblongata, cerebellum, hypothalamus, thalamus, and brainstem, are phylogenetically older with priority over other (younger, more anterior) regions for O_2 supply given their arguably more critical roles in maintaining cerebral homeostasis (Bailey, 2019b).

Pulmonary responses to hypoxia: peripheral and central respiratory chemoreflex

Throughout hypoxia, $\dot{V}_E$ increased progressively via O_2-mediated activation of the peripheral respiratory chemoreflex contributing to a gradual reduction in $P_{ET}CO_2$ and reciprocal elevation in $P_{ET}O_2$ (Forster *et al.*, 1971; Goldberg *et al.*, 1992; Sato *et al.*, 1992). However, peripheral respiratory chemoreflex-induced hyperventilation failed to fully compensate for the reduction in CDO_2 in hypoxia. The ventilatory response is also sensitive to changes in $P_{ET}CO_2$ regulated by the central respiratory chemoreflex (Forster *et al.*, 1971; Sato *et al.*, 1992; Fatemian & Robbins, 1998, 2001). In both normoxia and hypoxia, this has both fast (peripheral respiratory chemoreflex) and slow (central respiratory chemoreflex) components (Pedersen *et al.*, 1999). In the present study, we demonstrated that the $\dot{V}_E/P_{ET}CO_2$ response (central respiratory chemoreflex), was not affected by hypoxia. In contrast, the $\dot{V}_E/P_{ET}O_2$ response (peripheral respiratory chemoreflex) progressively increased in hypoxia, observations that confer with previous

studies highlighting a selective preference for the fast over the slow component (Fatemian & Robbins, 2001; Smith *et al.*, 2017). Importantly, the hypoxia-induced reduction in $P_{ET}O_2$ or CaO_2 was inhibited, changes that were independent of Hb since the latter remained invariant throughout. This finding indicates that the change in $P_{ET}O_2$ may be due to peripheral respiratory chemoreflex activation to offset the reduction in CDO_2. However, further research is warranted to fully understand the (cerebral) bioenergetic consequences of this response.

Cerebrovascular responses to hypoxia: CBF and CDO_2

In the present study, ICA, VA blood flow and gCBF increased in hypoxia, whereas ECA blood flow did not change consistent with previous research (Lewis *et al.*, 2014). An elevation in CBF compensates for systemic hypoxaemia and acts to preserve substrate (O_2 and glucose) delivery to the brain to maintain cerebral homeostasis (Bailey *et al.*, 2017a; Bailey, 2019a, b). ICA blood flow (a surrogate for anterior perfusion) increased, reaching its peak by the 2nd h of hypoxia before gradually decreasing there-on-in to values comparable with that observed in normoxia. The gCBF response profile was mostly attributable to changes in ICA blood.

In contrast, the initial increase in VA flow (surrogate for posterior CBF) was well maintained throughout hypoxia. ICA diameter increased whereas blood velocity gradually decreased throughout hypoxia, thus accounting for the observed progressive reduction in blood flow. In contrast, VA diameter remained invariant whereas blood velocity tended to increase throughout hypoxia. These findings highlight the differential contributions of diameter and velocity to the overall CBF response in the anterior and posterior cerebral circulation. Interestingly, although there is a significant difference in blood flow response between cerebral arteries, the CVC response profile was comparable for all arteries. Furthermore, the CVC responses to altered $P_{ET}O_2$ and $P_{ET}CO_2$ remained unchanged tentatively suggesting that the selective reduction in ICA blood flow may be prove the consequence of reduced ABP. However, we are unclear as to the specific mechanism(s) responsible for this differential regulation between arteries, though speculate it may be related to subtle anatomical differences in the cerebral pressure, strain, and shear stress phenotype in combination with possible differences in local blood

rheology (Calverley *et al.*, 2020) that although beyond the scope of the present study, warrant further examination.

These results confer in part with the findings of a previous study (Lewis *et al.*, 2014) that reported a more marked increase in VA compared to ICA blood flow during 6 h passive exposure to hypoxia. It has been suggested that selective site-specific changes in hypoxia-induced cerebral vasodilation may be subject to redox-regulation (Bailey *et al.*, 2017a; Bailey *et al.*, 2018) and associated differences in sensitivity to prostaglandins (Fan *et al.*, 2011; Harrell & Schrage, 2014), adenosine (Bowton *et al.*, 1988; Meno *et al.*, 1993) and sympathetic nerve activity (Curran-Everett *et al.*, 1992; Ainslie & Ogoh, 2010). More importantly, in the present study, ICA CDO_2 was lower in hypoxia despite the increase in ICA blood flow, implying that the observed hyperaemia was inadequate and could not fully compensate for arterial desaturation and lower prevailing CaO_2. In contrast, VA and ECA CDO_2 were well maintained throughout hypoxia.

Our previous studies (Ogoh *et al.*, 2008; Ogoh *et al.*, 2009) have highlighted the functional interaction between exercise-induced cardiopulmonary responses to CO_2 with CBF regulation for the maintenance of CO_2 homeostasis. However, these studies focused exclusively on the anterior cerebral circulation. We have consistently (Ogoh *et al.*, 2013b; Ogoh *et al.*, 2014b) demonstrated that poikilocapnic (i.e. hypocapnic) hypoxia does not increase anterior CBF (unchanged middle cerebral artery blood flow velocity or ICA blood flow) whereas increases have been consistently observed in response to isocapnic hypoxia. These findings indicate that hyperventilation-induced hypocapnia mediated by the peripheral respiratory chemoreflex antagonizes anterior cerebral vasodilation and consequent CDO_2 in hypoxia.

In contrast, acute hypoxia increased posterior CBF (VA blood flow) and CDO_2 (Ogoh *et al.*, 2013b). These findings suggest that the anterior cerebrovasculature is more sensitive to hypocapnia compared to the posterior circulation. Indeed, the human brain has evolved heightened sensitivity to $PaCO_2/H^+$ (more so than PaO_2) that extends throughout the cerebrovasculature, from the large extracranial and intracranial conduit and middle cerebral arteries through to the smallest pial arterioles and parenchymal vessels, prioritizing the buffering of brain tissue pH for stabilization of chemosensory and autonomic control at the level of the brainstem (Bailey *et al.*, 2017b).

Cerebrovascular CO_2 reactivity in the ECA and VA is lower than that of the ICA (Sato *et al.*, 2012b). It has been reported that acute hypoxia attenuated cerebrovascular CO_2 reactivity in the ICA (Ogoh *et al.*, 2014b), however, in the present study, there was no difference in CVC to changes in $P_{ET}CO_2$ between arteries, indicating that cerebrovascular CO_2 reactivity in the ICA exceeds that of ECA and VA.

Previous studies suggest that changes in $\dot{Q}$ contribute to CBF at rest and during exercise in normoxia (Ogoh *et al.*, 2005; Ogoh *et al.*, 2007). However, in the present study, the contribution of $\dot{Q}$ to VA and ECA blood flow remained unchanged, while that of ICA blood flow decreased throughout hypoxia. These findings suggest that the effect of the hypoxia-induced increase in $\dot{Q}$ on CBF and consequent CDO_2 regulation may be minimal.

AMS: mechanisms and pathogenesis

Traditionally, AMS has been considered a mild form of high-altitude cerebral oedema (HACE, the most malignant of all HA illness, oftentimes proving fatal) and that both syndromes share common pathophysiology linked by intracranial hypertension subsequent to vasogenic oedematous brain swelling, at opposing ends of a clinical continuum. An increase in intracranial pressure could potentially result in the mechanical stimulation of pain-sensitive unmyelinated fibres that reside within the trigeminovascular system, triggering the symptoms of headache (Bailey *et al.*, 2009a). However, more recent evidence suggests that functional impairment in cerebral 'venous outflow' at the level of the transverse venous sinus may prove the 'unifying' risk factor for AMS (Wilson *et al.*, 2013). In the present study, four out of eight participants (50 %) were diagnosed with clinical AMS. In these participants, the sustained elevation in ECA blood flow persisted throughout hypoxia and was proportional to symptom. These preliminary findings tentatively suggest that ECA vasodilatation may prove an alternative hemodynamic risk factor for AMS. However, we exercise caution and further research is warranted given that there was a clear uncoupling between the kinetics of ECA vasodilatation (early and sustained) and more progressive evolution of neurological sequelae. Rather than discount this hypothesis, it highlights the unavoidable challenges/limitations when attempting to correlate haemodynamic with symptomatic

data, complicated by markedly different onset kinetics.

We can only speculate regarding the 'potential' underlying mechanisms linking ECA vasodilation to the complex neurological sequelae that define AMS. Although controversial (Shevel, 2011), extracranial vasodilatation and distention of terminal branches of the ECA has been identified as a potential source of pain in migraine (Ray, 1940) which shares some phenotypical features with that of AMS (Broessner *et al.*, 2016). The ECA primarily supplies blood to the face, neck and meningeal branches with the middle meningeal artery (MMA) supplying the dura mater that is richly innervated by trigeminal sensory afferents (Mayberg *et al.*, 1984).

Subtle distension of the MMA in hypoxia such as that observed in the present study could potentially initiate the release of vasoactive peptides from activated dural trigeminal nerve endings that ultimately converge on the pain pathway (Khan *et al.*, 2019). Indeed, surgical cauterization of the terminal branches of the ECA (including the superficial temporal artery) is routinely employed in patients with migraine (Shevel, 2013). Furthermore, ECA 'flow diversion' (and subsequent AMS) may be viewed as a potentially adaptive neuroprotective response that serves to prevent cerebral hyperperfusion-mediated structural damage to the neurovascular unit, including the blood-brain barrier that is especially vulnerable especially in the face of impaired autoregulation and elevated cerebral oxidative-nitrosative stress (Bailey *et al.*, 2009b; Bailey *et al.*, 2009c). Clearly, more research is warranted in a 'higher-risk' setting taking advantage of rapid ascent to terrestrial high-altitude to confirm these laboratory-based observations and further establish its potential mechanistic bases.

Limitations

There are several limitations to the present study that warrant consideration. While we observed an apparent elevation in Hb, this likely reflected a dehydration-induced relative hemoconcentration since it was common to both inspirates. The relatively small sample size employed, despite a repeated measures design, limited our ability to detect differences in select variables owing to power constraints. We focused exclusively on young healthy men to avoid the potential cerebrovascular confounds associated with female sex hormones (Krejza *et al.*, 2013). While a recent study indicated that cerebral

perfusion at high-altitude did not change as a function of sex, this is not a universal finding justifying the inclusion of mixed sex sampling in future research (Liu *et al.*, 2016). Furthermore, we were not in a position to directly assess the central respiratory chemoreflex since we did not include a hyperoxic trial to eliminate any potential confounding contributions from the peripheral respiratory chemoreflex. In addition, we measured it in only six of eight participants in light of unforeseen technical complications. Equally, it is eminently plausible that the observed reduction in ICA CDO_2 may have been compensated by a reciprocal elevation in (cerebral) O_2 extraction as previously documented in a similar group of participants exposed to a comparable hypoxic stimulus (Bailey *et al.*, 2009c). However, given the absence of invasive arterio-jugular venous sampling, we were not in a position to formally address this. Finally, the peripheral respiratory chemoreflex was identified via steady-state changes in $\dot{V}_E$ during hypoxia. Thus, changes in $\dot{V}_E/P_{ET}CO_2$ and $\dot{V}_E/P_{ET}O_2$ need to be interpreted with caution since they reflect surrogate, albeit important, measures of central and peripheral respiratory chemoreflexes respectively.

In conclusion, the present study demonstrates that passive exposure to hypoxia alters CBF responses in the anterior and posterior cerebral circulation with CDO_2 better preserved in the latter. The observed heterogeneity was likely related to the differential CBF response to hypoxia rather than different sensitivities to O_2 and CO_2 mediated by the respiratory chemoreflex. Finally, ECA vasodilation may prove an alternative hemodynamic risk factor that predisposes to AMS. Collectively, these findings provide additional new insight into the compensatory mechanisms that serve to cerebral bioenergetics.

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Data availability statement

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Disclosure of conflict interests

No conflicts of interest, financial or otherwise, are declared by the authors.

Author Contributions

Author contributions: S.O., and B.S.S and D.M.B. conception and design of research; S.O., T.W., B.S.S., H.T., A.I., T.S.O., T.A.C., L.F., D.M.B. performed experiments; S.O., T.W., S.S., B.S.S., H.W., T.M. and D.M.B. analyzed data; S.O., T.W., B.S.S. and D.M.B. interpreted results of experiments; T.W. prepared figures; S.O. and D.M.B. drafted manuscript; all authors edited and revised manuscript; all authors approved final version of manuscript.

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782 **Table 1.** Cardiopulmonary responses during 7-hours exposure to normoxia and hypoxia

	condition	time							*P*-value			Effect size (η^2)		
		1h	2h	3h	4h	5h	6h	7h	condition	time	interaction	condition	time	interaction
HR (beat/min)	N	64 ± 27	63 ± 19	57 ± 13	59 ± 10	63 ± 24	55 ± 10	56 ± 9	0.051	0.887	0.081	0.066	0.017	0.104
	H	64 ± 12	64 ± 11	64 ± 11	68 ± 16	67 ± 9	72 ± 14	73 ± 11						
MAP (mmHg)	N	92 ± 13	93 ± 9	92 ± 5	94 ± 11	91 ± 7	96 ± 7	90 ± 14	**0.039**	0.878	0.595	0.166	0.019	0.026
	H	85 ± 9	85 ± 12	85 ± 12	83 ± 19	80 ± 13	77 ± 20	80 ± 12						
$\dot{Q}$ (L/min)	N	6.1 ± 2.3	6.1 ± 1.7	5.6 ± 1.1	5.9 ± 0.8	5.6 ± 0.8	5.5 ± 0.9	5.6 ± 0.9	**0.006**	0.615	0.095	0.138	0.026	0.086
	H	6.4 ± 1.1	6.4 ± 1.1	6.5 ± 1.0	6.8 ± 1.6	6.7 ± 0.9	7.2 ± 1.4	7.3 ± 1.1						
TVC (ml/min/mmHg)	N	66 ± 17	65 ± 15	60 ± 10	64 ± 11	62 ± 8	58 ± 9	65 ± 20	**0.004**	0.271	**0.015**	0.215	0.046	0.078
	H	76 ± 18*	77 ± 22*	77 ± 15*	88 ± 35	86 ± 20*	104 ± 52*	95 ± 25*						
RR (breath/min)	N	18 ± 6	18 ± 4	18 ± 3	18 ± 3	19 ± 2	18 ± 3	19 ± 4	0.121	0.110	0.244	0.040	0.100	0.059
	H	17 ± 6	18 ± 5	20 ± 7	19 ± 7	26 ± 12	22 ± 6	22 ± 5						
V_T (L)	N	1.0 ± 0.4	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.3	0.7 ± 0.2	0.7 ± 0.2	0.560	**0.005**	0.456	0.125	0.112	0.028
	H	1.1 ± 0.3	1.0 ± 0.2	0.9 ± 0.3	1.1 ± 0.2	0.9 ± 0.1	1.0 ± 0.3	1.0 ± 0.2						
$\dot{V}_E$ (L/min)	N	16.8 ± 6.2	15.5 ± 4.3	13.7 ± 3.1	13.8 ± 3.1	13.7 ± 3.9	12.8 ± 3.1	13.3 ± 3.5	**0.008**	0.415	0.134	0.232	0.028	0.083
	H	17.6 ± 3.0	17.9 ± 2.9	16.9 ± 5.7	18.9 ± 6.3	21.5 ± 6.5	19.1 ± 2.8	21.1 ± 3.4						
$P_{ET}O_2$ (mmHg)	N	95.6 ± 5.9	93.7 ± 4.1	91.8 ± 3.3	91.4 ± 3.4	91.1 ± 4.2	91.5 ± 2.5	90.9 ± 3.0	**< 0.001**	0.689	**<0.001**	0.972	< 0.001	0.005
	H	39.0 ± 5.4*	38.7 ± 3.7*	42.1 ± 3.3*	43.9 ± 4.3*	43.8 ± 4.2*	42.8 ± 5.3*	43.7 ± 3.3*						
$P_{ET}CO_2$ (mmHg)	N	41.5 ± 5.1	42.5 ± 4.8	41.4 ± 2.7	42.0 ± 2.8	41.6 ± 3.0	42.0 ± 2.6	41.5 ± 3.0	**0.001**	**0.005**	0.059	0.642	0.035	0.026
	H	34.9 ± 3.4	35.6 ± 3.4	33.3 ± 2.2	32.0 ± 2.7	30.5 ± 3.7	31.1 ± 3.2	30.7 ± 3.5						

SpO_2 (%)	N	98 ± 1	98 ± 1	97 ± 1	98 ± 1	98 ± 1	98 ± 0	98 ± 0	**<0.001**	0.278	0.134	0.825	0.009	0.015
	H	83 ± 4	83 ± 4	83 ± 4	87 ± 5	86 ± 3	85 ± 7	85 ± 3						
Hb (g/L)	N	141 ± 8	143 ± 8	148 ± 13	147 ± 10	146 ± 6	146 ± 9	146 ± 5	0.162	**0.001**	**0.034**	0.020	0.273	0.093
	H	140 ± 11	132 ± 6*	142 ± 11	147 ± 12†	146 ± 12†	145 ± 11†	146 ± 11†						
CaO_2 (ml/dL)	N	19.3 ± 1.1	19.5 ± 1.1	20.0 ± 1.7	19.9 ± 1.3	19.8 ± 0.8	19.8 ± 1.2	19.9 ± 0.7	**< 0.001**	**0.001**	**0.010**	0.508	0.075	0.038
	H	16.1 ± 1.5*	15.2 ± 1.3*	16.3 ± 1.6*	17.7 ± 2.0*	17.6 ± 1.7*†§	17.2 ± 2.1*	17.3 ± 1.2*						

Values are means ± SD. HR, heart rate; MAP, mean arterial pressure; $\dot{Q}$, cardiac output; TVC, total vascular conductance; RR, respiratory rate; V_T, tidal volume; $\dot{V}_E$, ventilation; $P_{ET}O_2$, end-tidal partial pressure of oxygen; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide; SpO_2; oxygen arterial saturation; Hb, hemoglobin concentration; CaO_2, arterial content of oxygen. $^*P < 0.05$ different from normoxia, $^\dagger P < 0.05$ different from 1 h, $^\S P < 0.05$ different from 3 h.

787 **Table 2** Relationships between site-specific cerebral oxygen delivery and change in respiratory/cerebrovascular responses

ICA CDO_2 (%)	MAP (%)		$P_{ET}O_2$ (%)		$P_{ET}CO_2$ (%)		$\dot{V}_E/\Delta P_{ET}O_2$ (L/min/mmHg)		$\dot{V}_E/\Delta P_{ET}CO_2$ (L/min/mmHg)	
Participant	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
1	0.00	1.00	**-0.89**	**0.01**	**0.79**	**0.04**	**0.82**	**0.02**	0.00	1.00
2	-0.11	0.82	0.61	0.15	-0.50	0.25	**-0.82**	**0.02**	-0.32	0.48
3	0.14	0.76	0.46	0.29	-0.61	0.15	-0.43	0.34	-0.25	0.59
4	0.07	0.88	0.36	0.43	-0.36	0.43	-0.43	0.34	-0.43	0.34
5	0.39	0.38	**-0.82**	**0.02**	0.64	0.12	**0.75**	**0.05**	0.29	0.53
6	0.43	0.34	-0.46	0.29	0.71	0.07	0.14	0.76	0.18	0.70
7	0.61	0.15	0.07	0.88	0.43	0.34	**0.86**	**0.01**	**0.86**	**0.01**
8	0.18	0.70	-0.32	0.48	0.29	0.53	0.46	0.29	0.04	0.94
VA CDO_2 (%)	MAP (%)		$P_{ET}O_2$ (%)		$P_{ET}CO_2$ (%)		$\dot{V}_E/\Delta P_{ET}O_2$ (L/min/mmHg)		$\dot{V}_E/\Delta P_{ET}CO_2$ (L/min/mmHg)	
Participant	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
1	0.00	1.00	-0.54	0.22	0.29	0.53	0.61	0.15	0.00	1.00
2	-0.04	0.94	-0.25	0.59	0.29	0.53	0.11	0.82	**-0.82**	**0.02**
3	0.04	0.94	0.14	0.76	-0.36	0.43	0.11	0.82	0.11	0.82
4	-0.46	0.29	0.39	0.38	**-0.75**	**0.05**	-0.29	0.53	-0.29	0.53
5	0.61	0.15	-0.54	0.22	0.57	0.18	0.21	0.64	-0.25	0.59
6	0.36	0.43	-0.29	0.53	-0.04	0.94	-0.14	0.76	-0.29	0.53

7	-0.25	0.59	0.18	0.70	-0.54	0.22	-0.07	0.88	-0.07	0.88
8	-0.54	0.22	0.61	0.15	**0.79**	**0.04**	-0.43	0.34	-0.54	0.22

ECA CDO_2 (%)	MAP (%)		$P_{ET}O_2$ (%)		$P_{ET}CO_2$ (%)		$\dot{\Delta}V_E/\Delta P_{ET}O_2$ (L/min/mmHg)		$\dot{\Delta}V_E/\Delta P_{ET}CO_2$ (L/min/mmHg)	
Participant	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
1	-0.18	0.70	-0.29	0.53	0.57	0.18	0.14	0.76	-0.04	0.94
2	0.00	1.00	-0.07	0.88	0.18	0.70	-0.25	0.59	-0.43	0.34
3	0.54	0.22	0.57	0.18	-0.79	0.04	-0.68	0.09	-0.36	0.43
4	**0.79**	**0.04**	0.14	0.76	0.04	0.94	-0.18	0.70	-0.18	0.70
5	0.29	0.53	0.14	0.76	-0.32	0.48	0.43	0.34	0.71	0.07
6	0.50	0.25	-0.11	0.82	0.50	0.25	0.11	0.82	0.04	0.94
7	-0.57	0.18	0.29	0.53	-0.39	0.38	-0.54	0.22	-0.54	0.22
8	0.39	0.38	-0.14	0.76	-0.43	0.34	0.25	0.59	0.50	0.25

788 r_s, Spearman's rank correlation coefficient.

789 **Table 3.** Cerebrovascular responses to normoxia and hypoxia

	condition	time							*P*-value			Effect size (η^2)		
		1h	2h	3h	4h	5h	6h	7h	condition	time	interaction	condition	time	interaction
Internal carotid artery														
Diameter (cm)	N	0.49 ± 0.05	0.50 ± 0.05	0.50 ± 0.05	0.50 ± 0.05	0.51 ± 0.05	0.50 ± 0.05	0.49 ± 0.04	**0.005**	**< 0.001**	**0.017**	0.075	0.124	0.037
	H	0.50 ± 0.04*	0.52 ± 0.04*	0.52 ± 0.04*	0.53 ± 0.04*	0.53 ± 0.04*	0.53 ± 0.05*	0.53 ± 0.04*						
Blood velocity (cm/s)	N	30.6 ± 4.7	29.9 ± 4.5	29.3 ± 5.1	30.0 ± 4.3	28.9 ± 3.9	29.5 ± 4.4	30.7 ± 3.9	0.284	**< 0.001**	**0.012**	0.006	0.291	0.229
	H	32.6 ± 6.0	32.3 ± 4.3*	31.3 ± 5.2*	27.8 ± 4.1‡§	26.7 ± 5.4	25.8 ± 5.6*‡§	27.2 ± 5.8*§						
Vertebral artery														
Diameter (cm)	N	0.36 ± 0.05	0.37 ± 0.05	0.37 ± 0.05	0.37 ± 0.05	0.37 ± 0.05	0.37 ± 0.06	0.37 ± 0.05	0.195	**0.010**	0.191	0.007	0.130	0.037
	H	0.36 ± 0.05	0.38 ± 0.05	0.38 ± 0.06	0.38 ± 0.06	0.38 ± 0.06	0.38 ± 0.06	0.38 ± 0.06						
Blood velocity (cm/s)	N	18.6 ± 3.0	18.0 ± 3.0	18.0 ± 3.1	18.1 ± 2.6	17.9 ± 2.6	17.4 ± 2.0	18.2 ± 2.5	0.078	**0.004**	0.073	0.092	0.094	0.050
	H	21.8 ± 2.6	20.7 ± 3.9	19.6 ± 3.1	18.7 ± 2.8	19.0 ± 2.9	19.4 ± 2.4	19.2 ± 2.6						
External carotid artery														
Diameter (cm)	N	0.45 ± 0.02	0.45 ± 0.01	0.45 ± 0.01	0.45 ± 0.02	0.45 ± 0.01	0.45 ± 0.00	0.45 ± 0.01	**0.001**	**0.005**	0.234	0.239	0.103	0.043
	H	0.45 ± 0.02	0.47 ± 0.02	0.47 ± 0.01	0.47 ± 0.01	0.46 ± 0.01	0.46 ± 0.01	0.47 ± 0.01						
Blood velocity (cm/s)	N	20.7 ± 2.5	20.6 ± 2.4	20.3 ± 2.9	19.6 ± 1.5	20.1 ± 2.9	20.1 ± 3.0	19.9 ± 2.1	0.813	**0.019**	0.596	0.002	0.078	0.030
	H	22.8 ± 4.6	21.3 ± 3.5	20.6 ± 5.6	19.7 ± 5.3	19.9 ± 5.7	19.2 ± 3.9	19.7 ± 3.2						

790 Values are means ± SD. $^{*}P < 0.05$ different from normoxia, $^{†}P < 0.05$ different from 1 h, $^{‡}P < 0.05$ different from 2 h, $^{§}P < 0.05$ different

791 from 3 h and $^{\#}P < 0.05$ from 4 h.

792

793 **Table 4.** Acute mountain sickness scores in normoxia and hypoxia

	condition	time							*P*-value			Effect size (η^2)		
		1h	2h	3h	4h	5h	6h	7h	condition	time	interaction	condition	time	interaction
LLS (points)	N	0.1 ± 0.4	0.4 ± 0.5	0.4 ± 0.5	0.5 ± 0.8	0.5 ± 0.8	0.5 ± 0.8	0.5 ± 0.8	**0.008**	**0.003**	**0.005**	0.257	0.182	0.133
	H	0.1 ± 0.4	1.6 ± 1.4*	1.6 ± 1.2*	2.4 ± 2.0*	2.4 ± 1.6*	4.1 ± 2.9*	4.4 ± 2.8*						
ESQ-C (a.u.)	N	0.00 ± 0.00	0.04 ± 0.05	0.04 ± 0.07	0.03 ± 0.04	0.03 ± 0.04	0.03 ± 0.04	0.03 ± 0.04	**0.046**	**0.030**	**0.047**	0.162	0.131	0.114
	H	0.00 ± 0.00	0.17 ± 0.25	0.14 ± 0.13*	0.27 ± 0.39	0.40 ± 0.47*	0.63 ± 0.82	0.74 ± 0.83*						
Headache score (mm)	N	0.0 ± 0.0	1.5 ± 2.8	3.9 ± 6.9	6.4 ± 10.9	5.9 ± 10.4	5.1 ± 10.3	4.5 ± 10.4	0.061	**0.017**	**0.002**	0.126	0.146	0.076
	H	1.3 ± 2.3	11.3 ± 17.1	11.3 ± 10.9	12.5 ± 14.1	13.1 ± 11.6	22.1 ± 21.4	26.9 ± 24.5						

794 Values are means ± SD. LLS, Lake Louise Score; ESQ-C, Environmental Symptoms Questionnaires-Cerebral symptoms. $^*P < 0.05$

795 different from normoxia.

796 **Table 5.** Relationships between cerebrovascular measures and acute mountain sickness

LLS (points)	ICA blood flow (ml/min)		ECA blood flow (ml/min)		VA blood flow (ml/min)	
Participant	r_s	*P*	r_s	*P*	r_s	*P*
1 (AMS+)	-0.185	0.526	0.244	0.4	0.421	0.134
2 (AMS-)	**0.626**	**0.017**	0.367	0.197	-0.41	0.145
3 (AMS-)	0.444	0.112	**0.637**	**0.014**	**0.598**	**0.024**
4 (AMS+)	**0.652**	**0.012**	**0.546**	**0.043**	**0.784**	**0.001**
5 (AMS+)	0.445	0.111	**0.591**	**0.026**	0.529	0.052
6 (AMS-)	-0.368	0.195	-0.43	0.125	**0.595**	**0.025**
7 (AMS+)	-0.096	0.743	**0.709**	**0.004**	0.331	0.248
8 (AMS-)	0.253	0.382	-0.253	0.382	0.506	0.065

ESQ-C (a.u.)	ICA blood flow (ml/min)		ECA blood flow (ml/min)		VA blood flow (ml/min)	
Participant	r_s	*P*	r_s	*P*	r_s	*P*
1 (AMS+)	-0.302	0.294	0.223	0.443	0.435	0.12
2 (AMS-)	**0.685**	**0.007**	0.464	0.095	-0.53	0.051
3 (AMS-)	0.464	0.095	**0.652**	**0.012**	**0.619**	**0.018**
4 (AMS+)	**0.674**	**0.008**	**0.603**	**0.023**	**0.752**	**0.002**
5 (AMS+)	0.432	0.123	**0.587**	**0.027**	0.543	0.045
6 (AMS-)	-0.421	0.134	-0.473	0.088	**0.563**	**0.036**
7 (AMS+)	-0.137	0.639	**0.771**	**0.001**	0.394	0.163
8 (AMS-)	0.081	0.783	0.445	0.111	-0.388	0.17

Headache score (mm)	ICA blood flow (ml/min)		ECA blood flow (ml/min)		VA blood flow (ml/min)	
Participant	r_s	*P*	r_s	*P*	r_s	*P*
1 (AMS+)	-0.409	0.147	-0.111	0.706	-0.115	0.695
2 (AMS-)	N/S	N/S	N/S	N/S	N/S	N/S
3 (AMS-)	0.449	0.107	**0.671**	**0.009**	**0.604**	**0.022**
4 (AMS+)	**0.711**	**0.004**	**0.631**	**0.016**	**0.829**	**<0.001**
5 (AMS+)	0.287	0.319	0.417	0.138	0.428	0.127

6 (AMS-)	-0.277	0.337	-0.351	0.218	**0.61**	**0.02**
7 (AMS+)	0.018	0.952	0.515	0.06	0.327	0.253
8 (AMS-)	0.107	0.715	**0.537**	**0.048**	-0.501	0.068

797 r_s, Spearman's rank correlation coefficient. AMS+/AMS- indicates participants
798 diagnosed with/without clinical (moderate-to-severe) acute mountain sickness.

Figure 1. Overview of the experimental protocol.

Figure 2. The percent changes in $P_{ET}O_2$, $P_{ET}CO_2$, and $\dot{V}_E$ from normoxia during 7 h hypoxia condition (n = 8). $P_{ET}O_2$, end-tidal partial pressure of oxygen; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide, $\dot{V}_E$, ventilation. †$P < 0.05$ different from 1 h, ‡$P < 0.05$ different from 2 h.

Figure 3. The sensitivities of central respiratory chemoreflex (A) ($\dot{V}_E$ response to hypercapnia under hypoxia condition, n = 6) and peripheral respiratory chemoreflex (B) ($\dot{V}_E$ response to hypoxia, n = 8). $\dot{V}_E$, ventilation; $P_{ET}O_2$, end-tidal partial pressure of oxygen; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide. The *P*-value refers to the one-way ANOVA main effect of the time-course changes of hypoxia. †$P<0.05$ different from 1 h.

Figure 4. The ICA, VA, ECA and global cerebral blood flow (A), CVC (B) and CDO_2 (C) response to normoxia and hypoxia (n = 8). ICA, internal carotid artery; VA, vertebral artery; ECA, external carotid artery; CVC, cerebral vascular conductance; CDO_2, cerebral oxygen delivery. *$P < 0.05$ different from normoxia, †$P < 0.05$ different from 1 h, ‡$P < 0.05$ different from 2 h, §$P < 0.05$ different from 3 h.

Figure 5. The ICA, VA, and ECA CVC response to changes in $P_{ET}O_2$ (A) or $P_{ET}CO_2$ (B) in hypoxia (n = 8). ICA, internal carotid artery; VA, vertebral artery; ECA, external carotid artery; CVC, cerebral vascular conductance; $P_{ET}O_2$, end-tidal partial pressure of oxygen; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide.

Figure 6. The percent change in Q (A) and total vascular conductance (B) in normoxia and hypoxia (n = 8). The change in the contribution of Q to ICA, ECA and VA blood flow during 7h hypoxia condition (C) (n = 8). $\dot{Q}$, cardiac output; TVC, total vascular conductance; ICA, internal carotid artery; VA, vertebral artery; ECA, external carotid artery. †$P < 0.05$ different from 1st h, ‡$P < 0.05$ different from 2nd h, §$P < 0.05$ different from 3rd h.

Figure 7. ECA blood flow response in participants with (AMS+, n = 4) and without AMS (AMS-, n = 4). ECA, external carotid artery; AMS, acute mountain sickness.