Alterations in cerebral blood flow and cerebrovascular reactivity during 14 days at 5050 m

Samuel J. E. Lucas[1,2], Keith R. Burgess[3], Kate N. Thomas[1], Joseph Donnelly[1], Karen C. Peebles[1], Rebekah A. I. Lucas[1,2], Jui-Lin Fan[1], James D. Cotter[2], Rishi Basnyat[4,5] and Philip N. Ainslie[6]

[1] *Department of Physiology and* [2] *School of Physical Education, University of Otago, Dunedin, New Zealand*
[3] *Department of Medicine, University of Sydney, Sydney, New South Wales, Australia*
[4] *Nepal International Clinic, Kathmandu, Nepal*
[5] *Himalayan Rescue Association, Kathmandu, Nepal*
[6] *Department of Health and Human Kinetics, Faculty of Health and Social Development, University of British Columbia Okanagan, Kelowna, British Columbia, Canada*

Non-technical summary Brain blood flow increases during the first week of living at high altitude. We do not understand completely what causes the increase or how the factors that regulate brain blood flow are affected by the high-altitude environment. Our results show that the balance of oxygen (O_2) and carbon dioxide (CO_2) pressures in arterial blood explains 40% of the change in brain blood flow upon arrival at high altitude (5050 m). We also show that blood vessels in the brain respond to increases and decreases in CO_2 differently at high altitude compared to sea level, and that this can affect breathing responses as well. These results help us to better understand the regulation of brain blood flow at high altitude and are also relevant to diseases that are accompanied by reductions in the pressure of oxygen in the blood.

Abstract Upon ascent to high altitude, cerebral blood flow (CBF) rises substantially before returning to sea-level values. The underlying mechanisms for these changes are unclear. We examined three hypotheses: (1) the balance of arterial blood gases upon arrival at and across 2 weeks of living at 5050 m will closely relate to changes in CBF; (2) CBF reactivity to steady-state changes in CO_2 will be reduced following this 2 week acclimatisation period, and (3) reductions in CBF reactivity to CO_2 will be reflected in an augmented ventilatory sensitivity to CO_2. We measured arterial blood gases, middle cerebral artery blood flow velocity (MCAv, index of CBF) and ventilation ($\dot{V}_E$) at rest and during steady-state hyperoxic hypercapnia (7% CO_2) and voluntary hyperventilation (hypocapnia) at sea level and then again following 2–4, 7–9 and 12–15 days of living at 5050 m. Upon arrival at high altitude, resting MCAv was elevated (up $31 \pm 31\%$; $P < 0.01$; *vs.* sea level), but returned to sea-level values within 7–9 days. Elevations in MCAv were strongly correlated ($R^2 = 0.40$) with the change in P_{aO_2}/P_{aCO_2} ratio (i.e. the collective tendency of arterial blood gases to cause CBF vasodilatation or constriction). Upon initial arrival and after 2 weeks at high altitude, cerebrovascular reactivity to hypercapnia was reduced ($P < 0.05$), whereas hypocapnic reactivity was enhanced ($P < 0.05$ *vs.* sea level). Ventilatory response to hypercapnia was elevated at days 2–4 ($P < 0.05$ *vs.* sea level, 4.01 ± 2.98 *vs.* $2.09 \pm 1.32\,l\,min^{-1}\,mmHg^{-1}$). These findings indicate that: (1) the balance of arterial blood gases accounts for a large part of the observed variability (~40%) leading to changes in CBF at high altitude; (2) cerebrovascular reactivity to hypercapnia and hypocapnia is differentially affected by high-altitude exposure and remains distorted during partial acclimatisation, and (3) alterations in cerebrovascular reactivity to CO_2 may also affect ventilatory sensitivity.

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Corresponding author S. Lucas: University of Otago, Dunedin 9054, New Zealand. Email: sam.lucas@otago.ac.nz

Abbreviations CBF, cerebral blood flow; CVCi, cerebral vascular conductance index; CVRi, cerebral vascular resistance index; [HCO_3^-], bicarbonate concentration; [Hct], haematocrit concentration; HR, heart rate; MAP, mean arterial blood pressure; MCAv, middle cerebral artery blood flow velocity; P_{aCO_2}, partial pressure of arterial CO_2; P_{aO_2}, partial pressure of arterial O_2; P_{ET,CO_2}, partial pressure of end tidal CO_2; P_{ET,O_2}, partial pressure of end tidal O_2; S_{aO_2}, arterial O_2 saturation; $\dot{V}_E$, ventilation.

Introduction

Upon ascent to high altitude, cerebral blood flow (CBF) rises substantially and then returns towards sea-level values within 1 week of exposure onset (Severinghaus *et al.* 1966*b*; Huang *et al.* 1987). The mechanisms underlying the regulation of CBF during exposure to high altitude are complex and depend partly on the degree of hypoxic stimulus itself and on the cerebrovascular sensitivity to hypoxia and CO_2 (Severinghaus, 2001; Ainslie & Ogoh, 2010). The partial pressures of arterial carbon dioxide (P_{aCO_2}) and oxygen (P_{aO_2}) play a major role in CBF regulation. A severe drop in P_{aO_2} (to <40–45 mmHg) produces cerebral vasodilatation (Cohen *et al.* 1967); however, whilst hypoxia *per se* is a cerebral vasodilator, the hypoxic-induced activation of peripheral chemoreceptor activity leads to hyperventilatory-induced lowering of P_{aCO_2} and subsequent cerebral vasoconstriction (Kety & Schmidt, 1948). The balance between these conflicting changes in arterial blood gases may be expected to alter during the process of acclimatisation (Severinghaus *et al.* 1966*b*; Ainslie & Ogoh, 2010). Surprisingly, a relationship between changes in CBF and the 'balance' or 'ratio' of arterial blood gases upon ascent to high altitude has not been reported. Upon arrival at high altitude, those individuals who exhibit a 'brisk' ventilatory response will have a higher P_{aO_2} and lower P_{aCO_2} than those individuals who have a 'blunted' ventilatory response. Although not established, it would seem reasonable to anticipate that changes in CBF at high altitude will be related to the balance of P_{aO_2}/P_{aCO_2} – a balance determined by an individual's ventilatory sensitivity to the hypoxic environment (Ainslie & Ogoh, 2010).

In addition to the critical importance of the prevailing arterial blood gas pressures, another factor that determines CBF is the relative degree of cerebral reactivity. Experimental studies that have examined the influence of exposure to high altitude (or hypoxia) on cerebral reactivity are limited and have variable results (Jansen *et al.* 1999; Blaber *et al.* 2003; Ainslie *et al.* 2007*b*; Ainslie & Burgess, 2008), and none have investigated what occurs during acclimatisation. Differences in experimental protocol (length of hypoxic exposure), method of assessing CBF reactivity, degree and type of hypoxic exposure (simulated *vs.* high altitude), and limited sample size may explain the differences in the observed CBF reactivity following exposure to high altitude. Recently, as assessed using a modified rebreathing method, we have reported that cerebrovascular reactivity and ventilatory sensitivity are both elevated upon initial (2–4 days) arrival at high altitude (Fan *et al.* 2010*a*). Whilst the rebreathing method clarifies some physiological effects associated with acute exposure to high altitude, it excludes the natural physiological influence of ventilatory sensitivity on cerebrovascular reactivity (Ainslie & Duffin, 2009). An alternative, integrated approach to explore the influence of ventilatory sensitivity on cerebrovascular reactivity is the use of a steady-state test. The steady-state method includes the influence of ventilatory-induced changes in P_{CO_2} gradients and CBF whereas the rebreathing method limits this influence (Fan *et al.* 2010*b*).

The implications of alterations in CBF and cerebrovascular reactivity to CO_2 are numerous (for review see Ainslie & Duffin, 2009), including a potential impact on ventilatory sensitivity (Xie *et al.* 2006; Fan *et al.* 2010*b*). For example, reductions in cerebrovascular CO_2 reactivity have been reported in the development of a heightened ventilatory response to CO_2 and unstable breathing pattern in patients with congestive heart failure (e.g. Xie *et al.* 2005) and obstructive sleep apnoea (e.g. Reichmuth *et al.* 2009; Burgess *et al.* 2010). Although it is acknowledged that peripheral chemoreceptors are crucial for the development of ventilatory acclimatisation at high altitude, changes in CO_2 sensitivity are also relevant (Dempsey & Forster, 1982) and therefore potentially influenced by alterations in cerebrovascular reactivity.

The three new hypotheses of this study were that: (1) the balance of arterial blood gases upon arrival at and across 2 weeks of living at 5050 m will closely relate to changes in CBF; (2) CBF reactivity to steady-state changes in CO_2 will be reduced across a 2 week acclimatisation period at 5050 m, and (3) the reductions in CBF reactivity to CO_2 will be reflected in an augmented ventilatory sensitivity to CO_2.

Methods

Participants

Seventeen participants (11 male and 6 female; mean ± S.D.: aged 31 ± 9 years; height, 1.7 ± 0.1 m; body mass, 69 ± 8 kg) volunteered for this study, which was approved by the Lower South Regional Ethics Committee of Otago and conformed to the standards set by the *Declaration of*

Helsinki. Participants were informed of the experimental procedures and possible risks involved in the study and written informed consent was obtained. All participants underwent full medical screening, including ECG and echo-cardiography assessment. Participants were not taking any medication, all were non-smokers, and none had any history of cardiovascular, cerebrovascular or respiratory disease. In addition, only two participants had previous high-altitude experience, which was >2 years previous to this expedition.

Experimental design and ascent protocol

Following full familiarisation with the experimental procedures (outlined below), the participants underwent two or four experimental trials: one at sea level (Dunedin, New Zealand; barometric pressure 755 ± 7 mmHg) and one or three repeated trials while living at high altitude (5050 m; the Ev-K2-CNR Pyramid Laboratory, Khumbu Valley, Nepal, at the base of Mt Everest; barometric pressure 413 ± 1 mmHg). The high-altitude trials were completed upon initial arrival (2–4 days) and then a sub-group of 10 participants were tested twice across a partial acclimatisation period of ∼2 weeks (at days 7–9 and 12–15). Sea-level testing was completed 2 weeks before arriving in Nepal. Participants spent 7 days at Kathmandu (∼1400 m) before flying to Lukla (2860 m). Participants then trekked to the Ev-K2-CNR Pyramid Laboratory over an 8 day period, which included rest days at Namche Bazar (3450 m) and Pheriche (4252 m).

With the exception of arterial blood gas sampling, which was conducted following 10 min supine rest, all experiments were performed with participants in a semi-recumbent position. Each experimental testing session included an arterial blood gas sample, instrumentation, a 5 min baseline data collection period, then 4 min of steady-state hyperoxic hypercapnia (7% CO_2–93% O_2) and 5 min of voluntary hyperventilation to induce steady-state hypocapnia. Hyperoxic hypercapnia was intentionally used in order to eliminate the influence of hypoxic-induced peripheral chemoreceptor activation at high altitude and acutely remove the influence of hypoxia on cerebrovascular tone. For hypocapnia, participants were instructed and given verbal feedback to increase ventilation (via depth and frequency) to lower and then maintain a partial pressure of end-tidal P_{CO_2} (P_{ET,CO_2}) at 22 ± 2 mmHg (sea level) and 17 ± 2 mmHg (5050 m). The order of each respiratory manipulation was randomised and full recovery was permitted between each trial. These tests were done between 15:00 and 19:00 at sea level and 12:00 and 19:00 at high altitude. The participants were instructed to avoid caffeine, alcohol and exercise within 12 h prior to the experimental testing.

This study was part of a larger research expedition and consequently participants took part in a number of studies conducted during the 2 weeks at the Pyramid Laboratory. However, none of the other studies involved exercise, and recovery time between testing sessions was managed to avoid potential confounding results (e.g. >48 h between all drug intervention studies). In addition, participants were given low-dose acetazolamide (125 mg bd) for the first 7 days of ascent as acute mountain sickness (AMS) prophylaxis, as recommended by others (Basnyat *et al.* 2006), to minimise the impact that AMS has on cerebrovascular and ventilatory responses (Jansen *et al.* 1999). Importantly, treatment was discontinued at day 7 to allow sufficient clearance time (i.e. >48 h) before experimental data were collected, since the reported half-life for acetazolamide is ∼10 h (Ritschel *et al.* 1998) and this low-dose quantity is reported to be 90–100% excreted within 24 h of administration (Richalet *et al.* 2005). AMS was assessed via the Lake Louise Questionnaire (LLQ; Roach *et al.* 1993) and Environmental Symptoms Questionnaire Cerebral Symptoms (ESQ-C; Sampson *et al.* 1983) scoring systems. Clinical diagnosis of AMS was determined from a LLQ score of ≥5 in the presence of a headache and an ESQ-C score ≥0.7, as recommended by others (Bailey *et al.* 2006).

Measurements of MCAv and arterial blood pressure. Blood flow velocity in the right middle cerebral artery (MCAv) was measured using a 2 MHz pulsed Doppler ultrasound system (DWL, Compumedics Ltd, Germany) using search techniques described elsewhere (Aaslid *et al.* 1982; Ainslie *et al.* 2005). The Doppler probe was secured with a plastic headband device (Spencer Technologies, USA) to maintain optimal insonation position and angle throughout the protocol. Beat-to-beat mean arterial blood pressure (MAP) and heart rate were also monitored using finger photoplethysmography (Finometer, Finapress Medical Systems, The Netherlands) and ECG, respectively. To ensure accurate measurements of MAP, right arm manual blood pressure measurements by auscultation were also made periodically to check and validate the automated recordings. From these cerebral and blood pressure data, indices of cerebrovascular resistance (pressure/flow) and conductance (flow/pressure) were calculated: $CVRi = MAP/MCAv_{mean}$ and $CVCi = MCAv_{mean}/MAP$, respectively.

Measurements and control of respiratory gas exchange. Participants breathed through a leak-free respiratory mask (Hans-Rudolph 7900 series, Kansas City, MO, USA) attached to a Y-shaped two-way non-rebreathing valve (Hans-Rudolph 7900) or through a mouthpiece (hyperventilation protocol), while wearing a nose clip. Inspiratory flow was measured using a heated pneumotach (Hans-Rudolph 3813) attached to the intake valve of the mask or to the mouthpiece, via a disposable filter. Pressures

of end-tidal CO_2 (P_{ET,CO_2}) and O_2 (P_{ET,O_2}) were sampled from the leak-free mask or from a needle inserted into the mouthpiece and measured using gas analysers (model CD-3A, AEI Technologies, Pittsburgh, PA; ML206 and ML240, ADInstruments). Ventilation (flow, tidal volume, frequency) and gas values were displayed in real time during testing (PowerLab, ADInstruments). Prior to each testing session, the pneumotachograph was calibrated using a 3-L syringe (Hans-Rudolph 5530) and the gas analysers were calibrated using known concentrations of O_2 and CO_2.

Cardiovascular and cerebrovascular haemodynamics and respiratory variables were measured continuously at 200 Hz using an analog-to-digital converter (Powerlab 16/30 ML880, Powerlab 8/30 ML870, ML240; ADInstruments) interfaced with a computer, and were subsequently analysed using commercially available software (Chart v.7, ADInstruments).

Blood gases. Arterial blood variables (pH, partial pressure of arterial O_2 (P_{aO_2}), partial pressure of arterial CO_2 (P_{aCO_2}), arterial O_2 saturation (S_{aO_2}), bicarbonate concentration [HCO_3^-], and haematocrit (Hct)) from the radial artery were obtained after 10 min supine rest using a 25-gauge needle into a preheparinised syringe. Following standardised calibration, all blood samples were analysed using an arterial blood-gas analysing system (NPT7 series, Radiometer, Copenhagen, Denmark).

Data analysis

Correction for hypocapnia. To normalise for the cerebral vasoconstrictive effects of hypocapnia at high altitude on resting measures, absolute MCAv values were also adjusted for changes in P_{aCO_2} (Bailey *et al.* 2009):

$$\text{Adjusted MCAv} = \frac{\text{measured MCAv during high altitude}}{[1 + (P_{aCO_2}\text{ during high altitude} - P_{aCO_2}\text{ baseline}) \times 0.03)]}$$

As used elsewhere (Bailey *et al.* 2009), this procedure allows for the estimation of the 'theoretical' changes in CBF in the hypoxia environment that would have occurred in the absence of the hypoxic-induced hyperventilation and related hypocapnia. In other words, it provides an estimation of the influence of hypoxia *per se* on CBF without the contrasting influence of hypocapnia.

Estimation of cerebrovascular reactivity. Cerebrovascular reactivity to CO_2 was expressed as the relative (%) change in MCAv per mmHg change in P_{ET,CO_2}. As used in other studies, MCAv and CVRi/CVCi were expressed as the relative (%) change from baseline to allow between-study comparisons and to reduce inter-individual variability that is unrelated to the experimental manipulation (Xie *et al.* 2005; Ainslie *et al.* 2007*b*).

Hypoxic cerebrovascular reactivity was calculated to assess the effect of the hypobaric hypoxia on changes in resting CBF from sea-level values. It was characterised in three complimentary ways: The relative change in MCAv divided by: (1) the change in S_{aO_2}; (2) the change in P_{aCO_2}; and (3) the change in P_{aO_2}/P_{aCO_2} ratio (i.e. $\Delta\text{MCAv}/\Delta S_{aO_2}$, $\Delta\text{MCAv}/\Delta P_{aCO_2}$ and $\Delta\text{MCAv}/(\Delta P_{aO_2}/P_{aCO_2})$, respectively). Since the ΔMCAv is relatively insensitive to hypoxia (at sea level), but sensitive to ΔP_{CO_2}, the use of the $\Delta\text{MCAv}/\Delta P_{aCO_2}$ index provides a means to assess the MCAv response to the related hypoxic-induced hypocapnia (Ainslie *et al.* 2007*a*).

Statistical analysis

Data were averaged over the final minute of baseline, hypocapnia and hypercapnia conditions. The time course of change for all measures was examined using linear mixed modelling (SPSS 17.0, SPSS Inc., IL, USA), which can be employed for unbalanced and mixed (between- and within-subjects factors) repeated measures designs, such as in the present study (Cnaan *et al.* 1997). Main and interaction effects of time (4 levels) and group (2 levels) were tested, with pair-wise comparisons (Bonferroni corrected) to identify differences between test-time. A planned comparison was also conducted to assess changes from sea-level measures. For all variables tested there was no difference between groups nor was there an interaction effect, therefore all participants were pooled into one group at sea level and upon initial arrival at high altitude. All data are expressed as mean ± S.D. Statistical inferences were based on an α-level of 0.05. The relationship between changes in MCAv and the blood gas balance (i.e. the P_{aO_2}/P_{aCO_2} ratio) at high altitude from sea-level values was tested using linear regression.

Results

Arterial blood gases, cerebrovascular and cardiovascular alterations at rest

Participants' arterial blood gases showed the expected initial respiratory alkalosis at high altitude, which was corrected with partial acclimatisation over the 14 days (Table 1). As anticipated, the lowest P_{aO_2} and the highest P_{aCO_2} were observed at days 2–4, although the subsequent recovery in P_{aO_2} was not statistically significant or as pronounced as that for S_{aO_2} (Table 1). Upon initial arrival at high altitude, MCAv was elevated (up 31 ± 31%, $P < 0.01$) compared to sea level, but returned to sea-level values by 7–9 days ($P = 0.30$; Table 1 and Fig. 1). Heart

Table 1. Arterial blood gases and cardiovascular and cerebrovascular function at sea level and across the initial 2 weeks following ascent to high altitude (5050 m)

Parameter	Sea level	High altitude		
		2–4 days	7–9 days	12–15 days
	($n = 17$)	($n = 17$)	($n = 10$)	($n = 10$)
Arterial blood gases				
P_{aO_2} (mmHg)	105 ± 11	44 ± 3*	46 ± 3*	48 ± 3*
P_{aCO_2} (mmHg)	42 ± 3	29 ± 3*	28 ± 2*	26 ± 2*†
pH	7.45 ± 0.04	7.47 ± 0.03*	7.45 ± 0.02	7.43 ± 0.03†
$[HCO_3^-]$ (mmol.L^{-1})	28.6 ± 3.1	21.3 ± 2.4*	19.0 ± 2.3*	17.1 ± 2.4*†
S_{aO_2} (%)	98.4 ± 0.5	79.9 ± 3.4*	82.9 ± 3.2*	85.5 ± 1.7*†
Hct (%)	45.0 ± 3.9	45.7 ± 3.9	49.4 ± 4.0*†	51.0 ± 5.0*†
P_{aO_2}/P_{aCO_2}	2.5 ± 0.4	1.5 ± 0.2*	1.7 ± 0.2*	1.9 ± 0.3*†
Cardiovascular function				
HR (beats min^{-1})	70 ± 11	80 ± 10*	80 ± 10*	78 ± 10*
MAP (mmHg)	81 ± 14	90 ± 15	83 ± 6	82 ± 9
Respiratory				
$\dot{V}_E$ (l min^{-1})	13.5 ± 1.8	16.3 ± 4.2*	18.8 ± 4.7*	22.4 ± 5.9*†
Cerebrovascular function				
MCAv (cm s^{-1})	66 ± 11	85 ± 17*	71 ± 10†	69 ± 10†
Systolic MCAv (cm s^{-1})	112 ± 20	130 ± 22*	114 ± 15	109 ± 16†
Diastolic MCAv (cm s^{-1})	43 ± 6	63 ± 14*	51 ± 9*†	49 ± 7*†
[1]Adjusted MCAv (cm s^{-1})	66 ± 11	138 ± 24*	118 ± 25*	125 ± 27*
CVR (mmHg cm s^{-1})	1.26 ± 0.36	1.07 ± 0.23	1.18 ± 0.18	1.24 ± 0.33
CVC (cm s^{-1} mmHg^{-1})	0.85 ± 0.24	0.97 ± 0.18	0.86 ± 0.12	0.85 ± 0.18

Values are means ± S.D. P_{aCO_2}, arterial P_{CO_2}; P_{aO_2}, arterial P_{O_2}; HCO_3^-, bicarbonate; S_{aO_2}, arterial O_2 saturation; Hct, haematocrit concentration; HR, heart rate; MAP, mean arterial blood pressure; $\dot{V}_E$, ventilation; MCAv, middle cerebral artery blood flow velocity; CVR, cerebral vascular resistance; CVC, cerebral vascular conductance. *$P < 0.05$: difference compared with sea level; †$P < 0.05$: difference compared with days 2–4 at high altitude. [1]Adjusted MCAv is measured MCAv during high altitude/[1 + (P_{aCO_2} during high altitude − P_{aCO_2} baseline) × 0.03)]; this procedure allows for the estimation of the 'theoretical' changes in cerebral blood flow in the hypoxia environment that would have occurred in the absence of the hypoxic-induced hyperventilation and related hypocapnia.

rate was elevated upon initial arrival at high altitude (up 11 ± 13 beats, $P < 0.01$) and remained elevated over the 14 days (Table 1). No significant changes in blood pressure, cerebral vascular resistance (CVR) and conductance (CVC) were observed across the acclimatisation period compared to sea level (all $P > 0.05$; Table 1).

The theoretical influence of hypoxia *per se* on MCAv decreased during the acclimatisation period, as shown by the CO_2-adjusted MCAv data in Table 1. This adaptation over time is consistent with the P_{aO_2}/P_{aCO_2} ratio (Table 1 and Fig. 1) and the reduced sensitivity to hypoxia (Table 2) across the partial acclimatisation.

Cerebrovascular and ventilatory reactivities to CO_2

Cerebrovascular reactivity to hypercapnia was lower on average at all three testing times at high altitude compared to sea level (Figs 3 and 4*B*); however, statistical significance was only reached at days 2–4 (down 26 ± 43%, $P = 0.02$; $n = 17$) and at days 12–15 (down 24 ± 30%, $P = 0.03$; $n = 10$). Cerebrovascular reactivity to hypocapnia was elevated at days 2–4 (up 153 ± 80%, $P < 0.01$), a response which persisted during the 2 weeks (up 70 ± 47% at days 12–15; Fig. 3). CVR for hypercapnia reactivity tests were greater (1.1 *vs.* 0.9 mmHg cm s^{-1}, $P = 0.03$) upon initial arrival compared to sea level, and remained elevated at days 7–9 (1.2 mmHg cm s^{-1}, $P < 0.01$ *vs.* sea level) and days 12–15 (1.1 mmHg cm s^{-1}, $P = 0.01$ *vs.* sea level). CVR for the hypocapnia tests was unchanged, $P = 0.74$) across the 2 week period from the sea-level measure (1.7 mmHg cm s^{-1}). Ventilatory response to hypercapnia was elevated upon arrival at high altitude (up 111 ± 127%, $P = 0.01$; 2.1 ± 1.3 and 4.0 ± 3.0 l min^{-1} mmHg^{-1}, sea level and days 2–4, respectively) and, on average, remained elevated across the 2 weeks living at high altitude, although not statistically so ($P > 0.05$ *vs.* sea level; 3.7 ± 1.9 and 3.2 ± 1.5 l min^{-1} mmHg^{-1}, days 7–9 and 12–15, respectively; Fig. 4*A*).

Discussion

The main novel findings of this study were that: (1) the balance of arterial blood gases, which are essentially determined by ventilatory sensitivity, accounted for a large part of the observed variability leading to changes in CBF at high altitude; (2) cerebrovascular reactivity to hypercapnia and hypocapnia were differentially affected and remained distorted during partial acclimatisation to high altitude; and (3) alterations in cerebrovascular reactivity to CO_2 may also affect ventilatory sensitivity. The following discussion considers the evidence, methodological assumptions and the relevance underlying the findings of this study.

Time course of changes in CBF at high altitude

Since first reported by Severinghaus and colleagues (1966*b*), the increased CBF upon initial exposure to high altitude and subsequent normalisation after a period of acclimatisation has been well described (Huang *et al.* 1987; Wolff, 2000; Ainslie & Ogoh, 2010). Importantly, the changes that we observed using transcranial Doppler (up 31%) were similar to those previously reported using different measuring techniques; e.g. Severinghaus *et al.* (1966*b*), using the Kety–Schmidt method, reported that CBF increased 24% on arrival (6–12 h) at high altitude (3810 m). The mechanisms underlying the regulation of CBF during exposure to hypoxia are influenced by the extent of the hypoxic stimulus itself and on the cerebrovascular sensitivity to hypoxia and CO_2 (Ainslie & Ogoh, 2010), acting in an interactive way (Severinghaus, 2001). The novel information that the current study contributes is the time course of the increase and subsequent normalisation of CBF at high altitude, along with the alterations in blood gas balance across this time course. For example, the low P_{aO_2}-to-P_{aCO_2} ratio at days 2–4 indicates a greater degree of hypoxic vasodilatation for a given hypocapnic vasoconstriction (Fig. 1) – this balance, prior to ventilatory acclimatisation, accounts for ∼40% of the initial increase in CBF upon arrival at high altitude (Fig. 2). Our data indicate that, within 7–9 days of living at high altitude, normalisation of CBF occurs due to hypoxic-induced elevations in ventilation (Table 1). This influence leads to a higher P_{aO_2}-to-P_{aCO_2} ratio, although only reaching statistical significance at days 12–15 ($P < 0.01$ *vs.* days 2–4), and therefore less hypoxic-induced dilatation and more hypocapnic-induced constriction in the cerebral circulation. Consistent with these findings are the adjusted MCAv data (Table 1) and the reduced hypoxic sensitivity at days 7–9 and 12–15 compared to initial arrival at high altitude (days 2–4, Table 2). These analyses reveal that the hypoxic vasodilatory contribution to CBF, independent of the changes in P_{aCO_2}, is greatest upon initial arrival at high altitude prior to ventilatory adjustments, and that the theoretical influence of hypoxia *per se* on MCAv is reduced during acclimatisation. Importantly, although the ventilatory acclimatisation relieves some of the hypoxaemia, it is not sufficient to remove the hypoxic vasodilatory effect altogether, since the normalised MCAv is still elevated compared to sea level (Table 1).

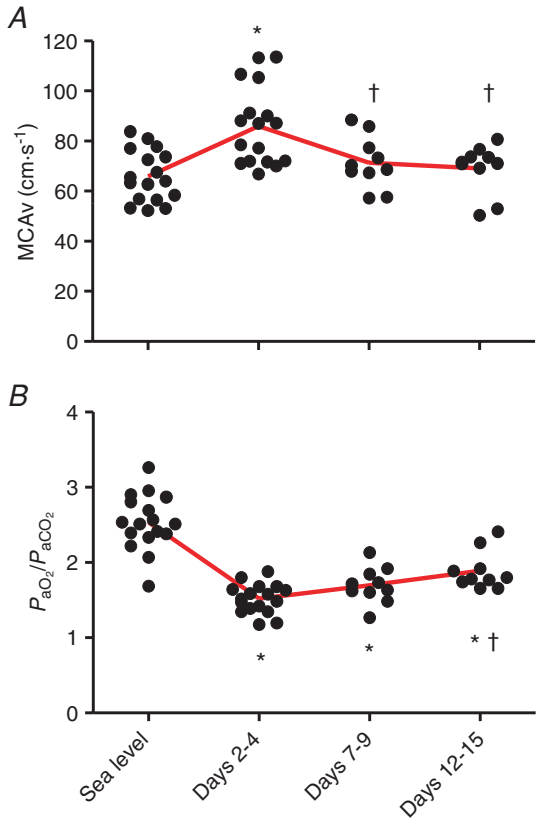

Figure 1. Cerebral blood flow velocity (MCAv, *A*) and ratio of arterial blood gases (P_{aO_2}/P_{aCO_2}, *B*) for eucapnic (room air) at sea level and upon initial arrival (2–4 days, $n = 17$) and following 7–9 days and 12–15 days ($n = 10$) of living at high altitude (5050 m)
*$P < 0.05$: difference compared with sea level; †$P < 0.05$: difference compared with days 2–4 at high altitude. The linear mixed model used revealed no difference between partial and full data sets nor was there an interaction effect; therefore, all participants were pooled into one group across the four time points.

Other mechanisms. We have shown for the first time that changes in the balance of arterial blood gases explain approximately 40% of the variance underlying the initial elevation in CBF at high altitude. It is acknowledged, however, that the CBF response upon arrival and during acclimatisation is affected by a myriad of other factors (reviewed in Ainslie & Ogoh, 2010). For example, CBF is increased by factors such as: neuronal (Golanov & Reis, 1996; Golanov *et al.* 2001); adenosine (Meno *et al.* 1993) and endothelium-derived NO (Faraci &

Table 2. Calculated hypoxic cerebrovascular reactivity[2] to ambient conditions at rest across the initial 2 weeks following ascent to high altitude (5050 m)

Parameter	Sea level	High altitude		
		2–4 days	7–9 days	12–15 days
Hypoxic cerebrovascular reactivity, $\%\Delta MCAv/\Delta S_{aO_2}$	—	1.7 ± 1.3	0.3 ± 0.8†	0.1 ± 1.1†
Hypoxic cerebrovascular reactivity, $\%\Delta MCAv/\Delta P_{aCO_2}$	—	2.9 ± 2.8	0.4 ± 0.9†	0.2 ± 1.0†
Hypoxic cerebrovascular reactivity, $\%\Delta MCAv/\Delta P_{aO_2}/P_{aCO_2}$	—	31.1 ± 20.9	4.3 ± 13.0†	1.7 ± 19.5†

†$P < 0.05$: difference compared with days 2–4 at high altitude. [2]Hypoxic cerebrovascular reactivity was characterised in three complimentary ways to assess the effect of the hypobaric hypoxia on changes in resting cerebral blood flow from sea-level values. These analyses revealed that the hypoxic vasodilatory contribution to cerebral blood flow is greatest upon initial arrival at high altitude prior to ventilatory adjustments, and that the theoretical influence of hypoxia *per se* on MCAv is reduced during acclimatisation.

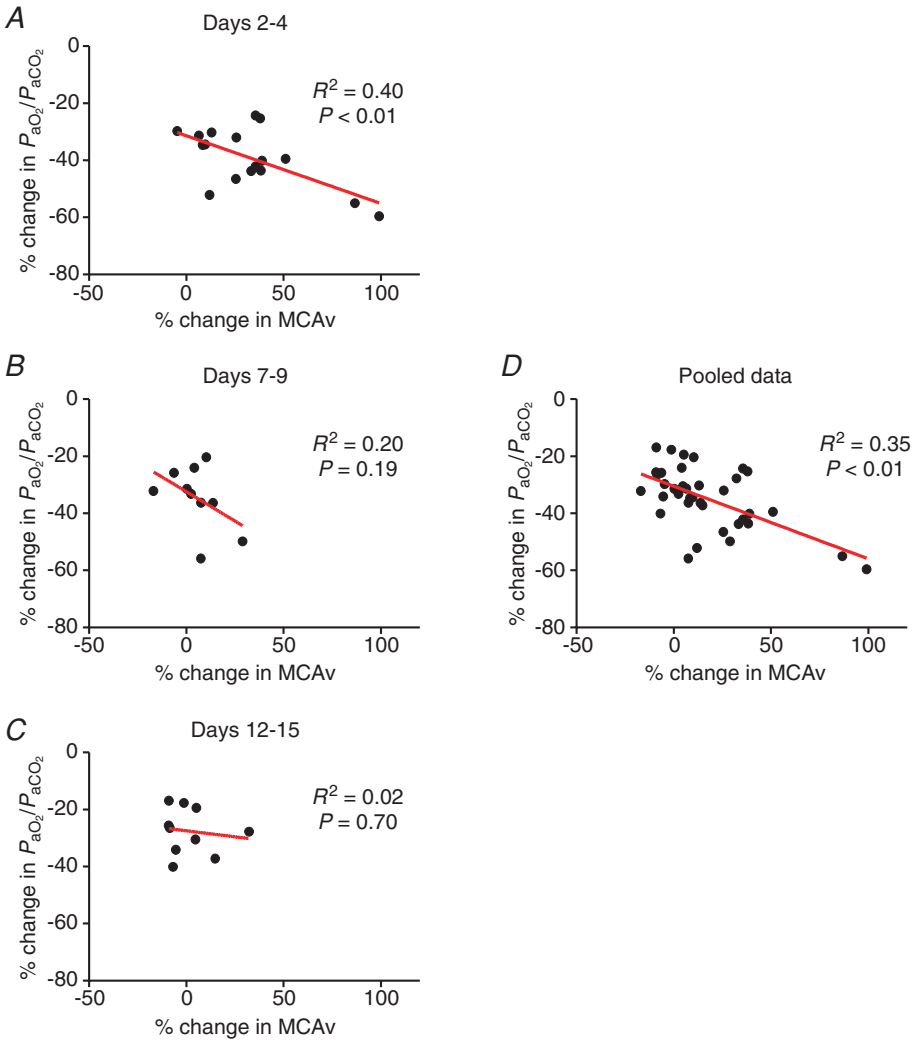

Figure 2. Relation between changes in MCAv and changes in the ratio of arterial blood gases
Relation between per cent changes in MCAv and changes in the ratio of arterial blood gases at sea level and upon initial arrival (*A*, 2–4 days, $n = 17$), following 7–9 days (*B*) and 12–15 days (*C*, $n = 10$) of living at high altitude (5050 m). Also presented is the pooled data set (*D*).

Brian, 1994; Bailey *et al.* 2009), whilst CBF is reduced due to a greater expression of superoxide (Toda *et al.* 2009) and noradrenaline (Rostrup, 1998), as well as increased haematocrit (Sorensen *et al.* 1974). The relative contributions of these factors in modulating CBF at high altitude is currently unknown and most probably change during acclimatisation, as we observed here for the influence of the arterial blood gas balance (Fig. 2). In addition, because of the apparent reductions in cerebral autoregulation at high altitude (Van Osta *et al.* 2005; Ainslie *et al.* 2007*b*; Jansen *et al.* 2007), which persist even following 4 weeks of acclimatisation (Iwasaki *et al.* 2010), subtle changes in blood pressure may have had some minor contribution to the time course changes in the MCAv. However, the lack of statistical changes we observed in blood pressure and subsequent relationship to CBF over this time indicates that its contribution is relatively minor compared to the balance of arterial blood gases.

Cerebrovascular reactivity

At sea level, steady-state cerebrovascular reactivity is more sensitive to increases in P_{CO_2} (i.e. hypercapnic reactivity) than decreases (i.e. hypocapnic reactivity) (Ide *et al.* 2003; Xie *et al.* 2005; Peebles *et al.* 2007). Data from the present study indicate that this relation is reversed upon initial arrival at high altitude, while following partial acclimatisation, hyper- and hypocapnia cerebrovascular reactivity were similar to one another and both significantly different to sea-level responses. Specifically, both steady-state hypercapnic and hypocapnic reactivity were altered (down 26% and up 153%, respectively; both $P < 0.05$ *vs.* sea level) upon arrival at 5050 m; responses that remained affected across a 2 week acclimatisation period (at days 12–15; down 24% and up 70%, respectively; both $P < 0.05$ *vs.* sea level; Figs 3 and 4). In addition, Fig. 5 shows the typical response across the 2 week acclimatisation for one individual, which clearly shows the reduced hypercapnic reactivity at high altitude compared to the sea-level response.

Jansen *et al.* (1999) has proposed that the augmented hypocapnic and reduced hypercapnic cerebrovascular reactivity at high altitude could potentially be explained

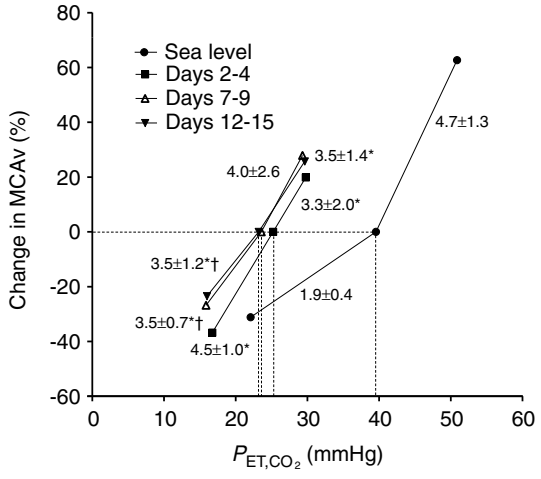

Figure 3. Changes in MCAv from baseline (eucapnia) during a 5 min voluntary hyperventilation (hypocapnia) and a 4 min steady-state hypercapnia (7% CO_2) at sea level and after 2–4, 7–9 and 12–15 days of living at high altitude (5050 m). Group cerebrovascular reactivity (% MCAv/mmHg CO_2; mean ± S.D.) for each slope (hypercapnia and hypocapnia) at sea level and during 2 weeks at high altitude.
*$P < 0.05$: difference compared with sea level; †$P < 0.05$: difference compared with days 2–4 at high altitude. These data indicate that cerebrovascular reactivity to hypercapnia and hypocapnia is differentially affected by high-altitude exposure and remains distorted during partial acclimatisation.

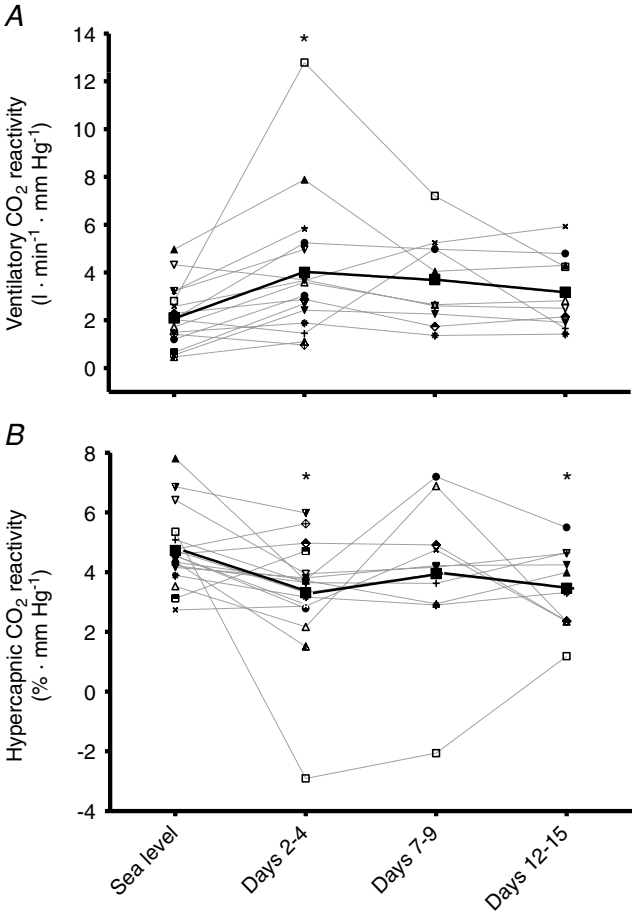

Figure 4. Individual ventilatory (*A*) and middle cerebral artery blood flow velocity (MCAv; *B*) responses to hypercapnia during wakefulness at sea level and after 2–4 days ($n = 17$), as well as at 7–9 days and 12–15 days of living at high altitude in a sub-group ($n = 10$) of participants
The mean of the group is also presented with an embolden line. *$P < 0.05$; difference compared with sea level. Participant symbols are consistent between each figure and statistical significance is not altered with the exclusion of the 'outlier' (open square symbol).

by a resetting of the total cerebral vasomotor reactivity (the sum of the fractional dilatation during hypercapnia and the fractional vasoconstriction during hypocapnia). The altered hypocapnic reactivity observed in the current study is consistent to that observed by Jansen *et al.* (1999), who reported a 71% higher reactivity in healthy newcomers to high altitude (4243 m) compared to a separate group of sea-level controls. Our data

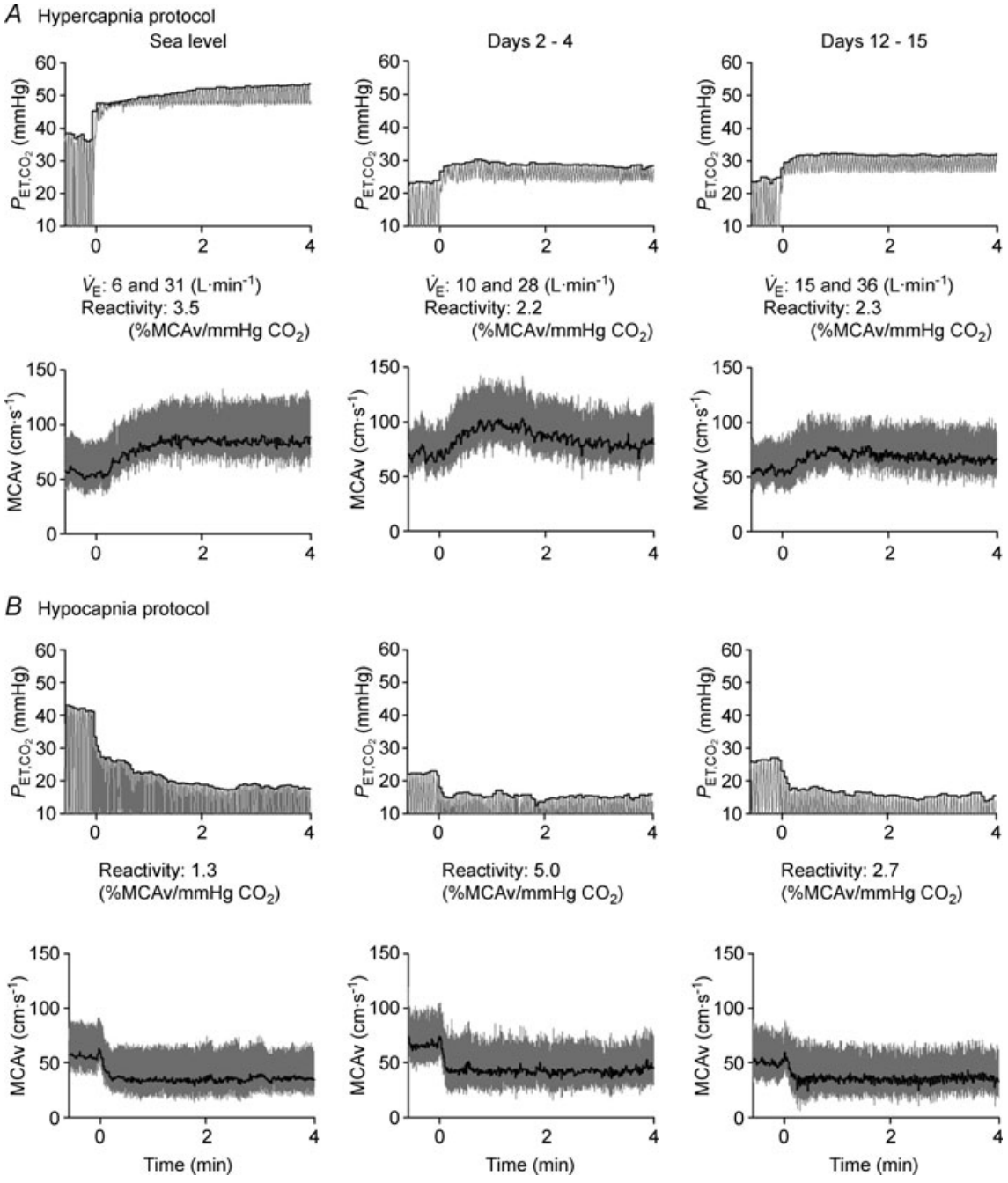

Figure 5. Representative recordings from one participant at sea level and after 2–4 days and 12–15 days of living at high altitude (5050 m)
A shows end-tidal P_{CO_2} (P_{ET,CO_2}, top row) and cerebral blood flow velocity (MCAv, bottom row) during a 4 min hypercapnic hyperoxia (7% CO_2–93% O_2) steady-state breathing protocol preceded by 30 s of room-air breathing. *B* shows these same data during the first 4 min of forced hyperventilation preceded by a 30 s of room-air breathing baseline. Ventilation (l min^{-1}) at rest for room-air breathing and during the hypercapnia reactivity protocol (*A*) is presented along with the calculated CO_2 reactivity (*A* and *B*) at each time point. These data illustrate the persistent reduction in hypercapnic reactivity and enhanced hypocapnic reactivity after 2 weeks of living at high altitude.

confirm this observation and importantly do so using a within-subject study design. Furthermore, the greater augmentation of hypocapnic reactivity and the *significant* reduction in hypercapnic reactivity observed in the present study indicates that higher altitudes than previously examined may alter cerebrovascular reactivity to a greater extent in healthy newcomers to high altitude. We had previously reported that the MCAv response to hypocapnia (voluntary hyperventilation) was blunted (Ainslie *et al.* 2007b) or unchanged (Ainslie & Burgess, 2008) at 3840 m, which conflicts with our present findings. One explanation for these differences is that the participants in our earlier study were tested following a 9 day ascent to altitudes >5000 m. Therefore, perhaps the descent (i.e. relative deacclimatisation), and related changes in arterial blood gases, from those at higher altitude are relevant in opposing the hypocapnia reactivity response. Recently, it has been suggested that augmented hypocapnic reactivity at high altitude could be an 'artefact' because of the influence of hyperventilation on elevating PaO_2 and thus reducing the hypoxic dilatory stimulus on the cerebrovasculature (Moller, 2010). There are two relevant points to this consideration. First, although PaO_2 within this range will have an effect on CBF dilation, it is unlikely that these changes would be solely responsible for our findings. For example, PaO_2 increases over this two-week time frame (Table 1) due to ventilatory acclimatisation, yet the heightened hypocapnic reactivity persists – a report consistent to that observed at a lower altitude (4243 m; Jansen, 1999). Second, regardless of the mechanism this augmented hypocapnic sensitivity is the physiological reality in cerebrovascular control: that is, a given drop in $PaCO_2$ at high altitude results in a greater drop in CBF. The implications of this maybe important in the pathophysiology of a range of cerebrovascular disorders at high altitude (e.g., AMS, CSA, cognitive impairment).

One potential explanation for the reduced dilatory capacity may be because of increased muscle sympathetic nervous activity (MSNA), consistently shown to occur at high altitude using a variety of direct and indirect MSNA measurement techniques (Saito *et al.* 1988; Duplain *et al.* 1999; Calbet, 2003; Hansen & Sander, 2003). However, an elevated MCAv response to hypercapnia during rebreathing protocols that we (Fan *et al.* 2010*a*) and others (Subudhi *et al.* 2010) have observed during hypobaric hypoxia (natural and simulated), along with the likelihood of an additional elevation in MSNA during such hypercapnic testing (Claassen *et al.* 2007), indicates that the mechanism for a reduced hypercapnia response during steady-state hypercapnia is more probably linked to changes in ventilation sensitivity. Such findings are consistent with the view that cerebral SNA is differentially controlled from MSNA (Ainslie & Tzeng, 2010).

Altered cerebrovascular CO_2 reactivity influences ventilatory sensitivity

In a parallel study to the present study, we observed an enhanced hypercapnic reactivity response during a modified rebreathing protocol (Fan *et al.* 2010*a*), whereas these steady-state data show an attenuated response. The critical difference between the steady-state and rebreathing methods for the assessment of hypercapnic cerebral reactivity is that the rebreathing protocol isolates the cerebrovascular reactivity response from any change in ventilatory sensitivity (i.e. as a consequence of living at high altitude), while the steady-state protocol includes this ventilatory response to P_{CO_2} (Ainslie & Duffin, 2009). The influence of changes in ventilation sensitivity at high altitude is illustrated in these steady-state cerebrovascular reactivity data. For example, one participant showed a negative change in MCAv with increased P_{CO_2} (Fig. 4*B*), which was accompanied by the greatest increase in ventilatory sensitivity at the same time points (Fig. 4*A*). Our findings of a reduction in cerebrovascular reactivity to CO_2 and reciprocal elevations in ventilatory sensitivity are broadly consistent with the findings of Xie *et al.* (2006) who, using indomethacin to impair hypercapnic cerebrovascular reactivity in healthy subjects at sea level, observed an augmented hypercapnia ventilatory response. The findings of Xie *et al.* highlighted the important role of cerebrovascular responsiveness in ventilatory control in the awake human. Thus, in addition to the powerful contribution of the peripheral chemoreceptors, the extent to which changes in cerebrovascular reactivity may also mediate the development of ventilatory acclimatisation and related breathing instability during sleep at high altitude warrants future consideration.

Implications

An enhanced CBF responsiveness to hypocapnia may be a contributing factor to the commonly, but not universally (Thomas *et al.* 2010), observed reductions in orthostatic tolerance during hypoxic conditions (Blaber *et al.* 2003). Importantly, the enhanced hypocapnic cerebrovascular response would increase an individual's risk of cerebral ischaemia during transient reductions in P_{aCO_2} and potentially may lead to impaired cognitive function during exposure to high altitude (Hornbein *et al.* 1989) and facilitate neurological injury (Wilson *et al.* 2009). The mechanisms underlying these reactivity responses as well as resting CBF changes warrant further study.

Tight control of the cerebrovascular CO_2 reactivity provides an important regulatory mechanism to minimise changes in brain $[H^+]$ at the level of the central chemoreceptor, and thereby stabilise breathing during fluctuating levels of chemical stimuli (Ainslie & Duffin, 2009). Consequently, a reduction in CBF hypercapnic responsiveness to CO_2 and/or an augmentation

in the hypocapnic range, as observed in the current study, may account in part for the development of periodic breathing commonly observed in newcomers to high altitude (Ainslie *et al.* 2007b). The causative link between altered cerebrovascular function and the occurrence of breathing instability at high altitude is incompletely understood and warrants further investigation.

Methodological considerations

Flow *vs.* velocity. CBFv has been shown to be a reliable and valid index of CBF (reviewed in Secher *et al.* 2008; Ainslie & Duffin, 2009) and our findings mirror the reported increases in CBF upon initial exposure to high altitude as determined by the direct Fick method (Severinghaus *et al.* 1966*b*; Roy *et al.* 1968; Moller *et al.* 2002). Moreover, since determinations of cerebrovascular reactivity are based on stimulus–response principles, absolute CBF values are not as important as reliable and repeatable recordings with short (beat-to-beat) time resolution. For these reasons, transcranial Doppler ultrasound is a well-suited technique for field-based research.

Potential influence of AMS on cerebrovascular CO_2 reactivity. Most participants did report mild symptoms of AMS on days 2–4 at high altitude; however, only 4/17 met clinical definitions of AMS, and correlations between AMS score and changes from sea-level values in either MCAv or hypercapnic cerebrovascular reactivity were negligible ($R^2 < 0.01$) and weak for hypocapnic cerebrovascular reactivity ($R^2 = 0.12$, $P = 0.17$). Furthermore, significance in our results was not altered with the removal of these four participants at this time point; as such, all participant data were included. Moreover, all participants were free of any AMS symptoms at days 12–15, a time point where the altered reactivity responses persisted; thus, we are confident that our observed changes upon arrival at 5050 m were not confounded by mild AMS. It should be noted that the current study intentionally avoided the peak AMS period, which may explain the lack of significant change in blood pressure and pressure/flow indices. Thus, our observations of increased CBF upon arrival at high altitude are likely to underestimate what occurs with AMS. Given the higher prevalence of AMS (and high altitude cerebral oedema) in the unacclimatised visitor to high altitude (Hackett & Roach, 2001), further investigation is warranted to clarify the impact of AMS on cerebrovascular responses.

Assessment of cerebrovascular CO_2 reactivity. We used a steady-state open circuit method for measuring MCAv and ventilatory CO_2 responsiveness. Accordingly, we maintained a P_{CO_2} gradient between the blood and the brain. This approach closely mimics 'normal' physiological conditions, especially during sleep when P_{aCO_2} is transiently increased secondary to hypoventilation or apnoea. A hyperoxic background was used during the CO_2 response tests to reduce the likely influence of peripherial chemoreflex drive. Although we acknowledge that hyperoxia alone may induce some mild cerebral vasoconstriction (Ainslie *et al.* 2008), this would be unlikely to affect our results.

It should also be noted that our 'steady-state' determination of CBF and ventilatory CO_2 sensitivity was obtained using only two data points (baseline and 7% CO_2). Nevertheless, the steady-state CBF and $\dot{V}_E$ CO_2 sensitivity observed in the present study were comparable to that reported by Xie *et al.* (2006), who used four steady-state data points (baseline, 2%, 4% and 6% CO_2). In addition, because of a 'resetting' of the respiratory chemoreflex to a lower P_{aCO_2} at high altitude (Dempsey & Forster, 1982), and in view of the linear CBF and $\dot{V}_E$ responses to CO_2 within this physiological range, we feel that our findings based on two data points would not be a confounding factor.

Finally, it is important to note that the absolute change in P_{ET,CO_2} (and therefore $[H^+]$ at the central chemoreceptors) at high altitude was less than that at sea level (6–7 mmHg *vs.* 13 mmHg). However, given the logarithmic relation between P_{CO_2} and pH (i.e. $\Delta pH = \Delta \log P_{CO_2}$), any change in P_{CO_2} at high altitude will result in a greater pH change compared with the equivalent sea-level P_{CO_2} and thus the lower P_{aCO_2} at high altitude may overestimate the hypercapnia responses (Severinghaus *et al.* 1966*a*). Nevertheless, for reasons of comparison with previous studies (which did not correct for this), together with the fact that corrected responses show a *greater* impairment in hypercapnia reactivity during the steady-state protocol and therefore do not change our overall findings, we did not present these corrected data.

Conclusion

The balance of arterial blood gases accounted for a large part of the observed variability leading to changes in CBF at high altitude. High altitude exposure alters the cerebrovascular reactivity to hypercapnia and hypocapnia differentially and they remained distorted during partial acclimatisation. These alterations in cerebrovascular reactivity to CO_2 may, in part, also affect ventilatory sensitivity and breathing stability.

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Author contributions

All the listed authors were involved in: (1) the conception and design, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published.

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