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

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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 (O2) and carbon dioxide (CO2) 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 CO2 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 CO2 will be reduced following this 2 week acclimatisation period, and (3) reductions in CBF reactivity to CO2 will be reflected in an augmented ventilatory sensitivity to CO2. We measured arterial blood gases, middle cerebral artery blood flow velocity (MCAv, index of CBF) and ventilation (VE) at rest and during steady-state hyperoxic hypercapnia (7% CO2) 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 ± 31%; P < 0.01; vs. sea level), but returned to sea-level values within 7–9 days. Elevations in MCAv were strongly correlated (R2 = 0.40) with the change in Pao2/Paco2 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 ± 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 CO2 may also affect ventilatory sensitivity.
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. 1966b; 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 CO₂ (Severinghaus, 2001; Ainslie & Ogoh, 2010). The partial pressures of arterial carbon dioxide (PₐCO₂) and oxygen (PₐO₂) play a major role in CBF regulation. A severe drop in PₐO₂ (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 PₐCO₂ 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. 1966b; 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 PₐO₂ and lower PₐCO₂ 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 PₐO₂/PₐCO₂ – 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. 2007b; 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. 2010a). 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 PₐCO₂ gradients and CBF whereas the rebreathing method limits this influence (Fan et al. 2010b).

The implications of alterations in CBF and cerebrovascular reactivity to CO₂ are numerous (for review see Ainslie & Duffin, 2009), including a potential impact on ventilatory sensitivity (Xie et al. 2006; Fan et al. 2010b). For example, reductions in cerebrovascular CO₂ reactivity have been reported in the development of a heightened ventilatory response to CO₂ 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 CO₂ 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 CO₂ will be reduced across a 2 week acclimatisation period at 5050 m, and (3) the reductions in CBF reactivity to CO₂ will be reflected in an augmented ventilatory sensitivity to CO₂.

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

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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% CO₂–93% O₂) 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 CO₂ (P_{ET,CO₂}) 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 (Ricahe 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 CVGi = 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\textsubscript{2} (P\textsubscript{ET,CO\textsubscript{2}}) and O\textsubscript{2} (P\textsubscript{ET,O\textsubscript{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\textsubscript{2} and CO\textsubscript{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 \textsubscript{O\textsubscript{2}} (P\textsubscript{aO\textsubscript{2}}), partial pressure of arterial \textsubscript{CO\textsubscript{2}} (P\textsubscript{aCO\textsubscript{2}}), arterial \textsubscript{O\textsubscript{2}} saturation (S\textsubscript{aO\textsubscript{2}}), bicarbonate concentration [HCO\textsubscript{3}\textsuperscript{−}], 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 vasconstrictive effects of hypocapnia at high altitude on resting measures, absolute MCA\textsubscript{v} values were also adjusted for changes in P\textsubscript{aCO\textsubscript{2}} (Bailey \textit{et al.} 2009):

Adjusted MCA\textsubscript{v} = \text{measured MCA\textsubscript{v} during high altitude} / [1 + \left(\frac{P\textsubscript{aCO\textsubscript{2}} \text{ during high altitude} - P\textsubscript{aCO\textsubscript{2}} \text{ baseline}}{0.03}\right)]

As used elsewhere (Bailey \textit{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\textsubscript{2} was expressed as the relative (%) change in MCA\textsubscript{v} per mmHg change in P\textsubscript{ET,CO\textsubscript{2}}. As used in other studies, MCA\textsubscript{v} and CVR\textsubscript{I}/CVC\textsubscript{I} 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 \textit{et al.} 2005; Ainslie \textit{et al.} 2007b).

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 MCA\textsubscript{v} divided by: (1) the change in S\textsubscript{aO\textsubscript{2}}; (2) the change in P\textsubscript{aCO\textsubscript{2}}; and (3) the change in P\textsubscript{aO\textsubscript{2}}/P\textsubscript{aCO\textsubscript{2}} ratio (i.e. ∆MCA\textsubscript{v}/∆S\textsubscript{aO\textsubscript{2}}, ∆MCA\textsubscript{v}/∆P\textsubscript{aCO\textsubscript{2}} and ∆MCA\textsubscript{v}/(∆P\textsubscript{aO\textsubscript{2}}/P\textsubscript{aCO\textsubscript{2}}), respectively). Since the ∆MCA\textsubscript{v} is relatively insensitive to hypoxia (at sea level), but sensitive to ∆P\textsubscript{CO\textsubscript{2}}, the use of the ∆MCA\textsubscript{v}/∆P\textsubscript{aCO\textsubscript{2}} index provides a means to assess the MCA\textsubscript{v} response to the related hypoxic-induced hypocapnia (Ainslie \textit{et al.} 2007a).

**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 \textit{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 MCA\textsubscript{v} and the blood gas balance (i.e. the P\textsubscript{aO\textsubscript{2}}/P\textsubscript{aCO\textsubscript{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\textsubscript{aCO\textsubscript{2}} and the highest P\textsubscript{aO\textsubscript{2}} were observed at days 2–4, although the subsequent recovery in P\textsubscript{aO\textsubscript{2}} was not statistically significant or as pronounced as that for S\textsubscript{aO\textsubscript{2}} (Table 1). Upon initial arrival at high altitude, MCA\textsubscript{v} 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
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 \( P_{acO_2} / P_{acO_2} \) 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 4B); 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 hypcapnia 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 hypcapnia 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 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 min\(^{-1} \) mmHg\(^{-1} \), days 7–9 and 12–15, respectively; Fig. 4A).
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 CO₂ 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 (1966b), 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. (1966b), 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 CO₂ (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 \( P_{aO2}/P_{aCO2} \) 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 \( \sim 40\% \) 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 \( P_{aO2}/P_{aCO2} \) 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 \( P_{aCO2} \), is greatest upon initial arrival at high altitude prior to ventilatory adjustments, and that the theoretical influence of hypoxia \( \text{per se} \) 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).

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 to ambient conditions at rest across the initial 2 weeks following ascent to high altitude (5050 m)

| Parameter                                      | Sea level | 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, \% \(\Delta MCAv/\Delta P_{aO_2}/\Delta P_{aCO_2}\) | —         | 31.1 ± 20.9| 4.3 ± 13.0| 1.7 ± 19.5†|

†P < 0.05: difference compared with days 2–4 at high altitude. ²Hypoxic 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).

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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. 2007b; 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...
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 $P_{\text{ET}, \text{CO}_2}$ ($P_{\text{ET}, \text{CO}_2}$, top row) and cerebral blood flow velocity (MCAv, bottom row) during a 4 min hypercapnic hyperoxia (7% $\text{CO}_2$–93% $\text{O}_2$) steady-state breathing protocol preceded by 30 s of room-air breathing. Ventilation ($l \text{ min}^{-1}$) at rest for room-air breathing and during the hypercapnia reactivity protocol (A) is presented along with the calculated $\text{CO}_2$ 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 PaO2 and thus reducing the hypoxic dilatory stimulus on the cerebrovasculature (Moller, 2010). There are two relevant points to this consideration. First, although PaO2 within this range will have an effect on CBF dilatation, it is unlikely that these changes would be solely responsible for our findings. For example, PaO2 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 PaCO2 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. 2010a) and others (Subudhi et al. 2010) have observed during hypocapnic 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 CO₂ 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. 2010a), 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\textsubscript{CO₂} (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\textsubscript{CO₂} (Fig. 4B), which was accompanied by the greatest increase in ventilatory sensitivity at the same time points (Fig. 4A). Our findings of a reduction in cerebrovascular reactivity to CO₂ 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\textsubscript{aCO₂} 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 CO₂ 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 CO₂ 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. 1966b; 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 CO₂ 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 CO₂ reactivity. We used a steady-state open circuit method for measuring MCAv and ventilatory CO₂ 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 CO₂ response tests to reduce the likely influence of peripheral 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 CO₂ sensitivity was obtained using only two data points (baseline and 7% CO₂). Nevertheless, the steady-state CBF and $V_E$ CO₂ 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% CO₂). 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 $V_E$ responses to CO₂ 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. $\Delta pH = \Delta logP_{CO_2}$), any change in $P_{aCO_2}$ 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. 1966a). 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 CO₂ 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.

Acknowledgements
This study was carried out within the framework of the Ev-K2-CNR Project in collaboration with the Nepal Academy of Science and Technology as foreseen by the Memorandum of Understanding between Nepal and Italy, and thanks to contributions from the Italian National Research Council and the Italian Ministry of Foreign Affairs. We extend our thanks to ADInstruments and Compumedics Ltd for the use of their laboratory equipment and Professor Greg Atkinson for his advice on the statistical analysis. This study was supported by the Otago Medical Research Foundation, SPARC New Zealand, The Peninsula Health Care p/l and Air Liquide p/l.