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Effects of acetazolamide on cerebrovascular function and breathing stability at 5050 m

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Key points

- Acetazolamide improves breathing stability during sleep in newcomers to high altitude, but the mechanism remains unclear.
- We examined the effects of a single I.V. dose of acetazolamide on brain vascular function and breathing at sea level and following 7 days at high altitude (5050 m).
- We demonstrated that acute I.V. acetazolamide at high altitude enhances the brain blood flow response to changes in CO_2 and improves breathing stability.
- We speculate that the enhanced brain blood flow responses following acetazolamide ingestion may account for the well-documented acetazolamide-induced improvement in abnormal breathing at high altitude.

Abstract One of the many actions of the carbonic anhydrase inhibitor, acetazolamide (ACZ), is to accelerate acclimatisation and reduce periodic breathing during sleep. The mechanism(s) by which ACZ may improve breathing stability, especially at high altitude, remain unclear. We tested the hypothesis that acute I.V. ACZ would enhance cerebrovascular reactivity to CO_2 at altitude, and thereby lower ventilatory drive and improve breathing stability during wakefulness. We measured arterial blood gases, minute ventilation ($\dot{V}_E$) and middle cerebral artery blood flow velocity (MCAv) before and 30 min following ACZ administration (I.V. 10 mg kg^{-1}) in 12 healthy participants at sea level and following partial acclimatisation to altitude (5050 m). Measures were made at rest and during changes in end-tidal P_{CO_2} and P_{O_2} (isocapnic hypoxia). At sea level, ACZ increased resting MCAv and its reactivity to both hypocapnia and hypercapnia ($P < 0.05$), and lowered resting $\dot{V}_E$, arterial O_2 saturation (S_{a,O_2}) and arterial P_{O_2} (P_{a,O_2}) ($P < 0.05$); arterial P_{CO_2} (P_{a,CO_2}) was unaltered ($P > 0.05$). At altitude, ACZ also increased resting MCAv and its reactivity to both hypocapnia and hypercapnia (resting MCAv and hypocapnia reactivity to a greater extent than at sea level). Moreover, ACZ at altitude elevated P_{a,CO_2} and again lowered resting P_{a,O_2} and S_{a,O_2} ($P < 0.05$). Although the $\dot{V}_E$ sensitivity to hypercapnia or isocapnic hypoxia was unaltered following ACZ at both sea level and altitude ($P > 0.05$), breathing stability at altitude was improved (e.g. lower incidence of ventilatory oscillations and variability of tidal volume; $P < 0.05$). Our data indicate that I.V. ACZ elevates cerebrovascular reactivity and improves breathing stability at altitude, independent of changes in peripheral or central chemoreflex sensitivities. We speculate

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that P_{a,CO_2}-mediated elevations in cerebral perfusion and an enhanced cerebrovascular reactivity may partly account for the improved breathing stability following ACZ at high altitude.

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Abbreviations ACZ, acetazolamide; AMS, acute mountain sickness; CBF, cerebral blood flow; CVCi, cerebrovascular conductance index; MAP, mean arterial blood pressure; MCAv, middle cerebral artery blood flow velocity.

Introduction

The stability of breathing rhythm is thought to depend strongly on the 'CO_2 reserve', i.e. the difference between the eupnoeic pressure of CO_2 in arterial blood (P_{a,CO_2}) and the hypocapnic P_{a,CO_2} threshold for apnoea (see Dempsey (2005) for review). The CO_2 reserve is reduced when breathing hypoxic air because of increased ventilation (due to the peripheral chemoreflex activation) lowering P_{a,CO_2}, thereby increasing the risk of periodic breathing during sleep (Xie *et al.* 2001, 2006). Ascent to high altitude in newcomers often leads to unstable breathing during both wakefulness (Brusil *et al.* 1980; Waggener *et al.* 1984) and sleep (Sutton *et al.* 1979, 1980; Lahiri *et al.* 1983; Normand *et al.* 1990; Lahiri & Data, 1992). Typically an oscillatory pattern of breathing can be distinguished, especially during sleep, with changes in tidal volume that can develop into periodic breathing with central apnoea periods. This development of breathing instability is probably due to a greater increase in the hypoxic ventilatory response associated with high altitude-induced hypoxaemia compared with the increase in background ventilatory drive, resulting in a reduced CO_2 reserve (Khoo *et al.* 1982; Dempsey, 2005). The carbonic anhydrase inhibitor acetazolamide (ACZ) diminishes breathing pattern disturbances upon ascent to high altitude, especially during sleep (Weil *et al.* 1978; Sutton *et al.* 1979, 1980). At sea level, ACZ increases the CO_2 reserve in both humans (Teppema *et al.* 2010) and anaesthetised cats (Teppema *et al.* 2001). However, since both the peripheral (Hackett *et al.* 1987) and central chemoreflex sensitivities (Burki *et al.* 1992) appear to be preserved following ACZ at high altitude, it seems unlikely that a blunted ventilatory drive would account for the increase in CO_2 reserve. Accordingly, the prophylactic mechanisms of action by which ACZ improves breathing stability during wakefulness and sleep remain poorly understood.

One potential mechanism by which ACZ may improve the CO_2 reserve is via an effect on cerebral blood flow (CBF). Several studies have demonstrated a link between resting CBF and its response to CO_2 (termed cerebrovascular CO_2 reactivity), and the CO_2 reserve (Xie *et al.* 2005, 2009). For example, pharmacologically induced reductions in resting CBF and cerebrovascular CO_2 reactivity reduce the CO_2 reserve during sleep (Xie *et al.* 2009) and cause breathing instability during wakefulness (Fan *et al.* 2010*b*). It has been well documented that, at sea level, acute I.V. administration of ACZ rapidly elevates resting CBF (Ehrenreich *et al.* 1961; Hauge *et al.* 1983; Lassen *et al.* 1987; Jensen *et al.* 1990) without altering cerebral metabolism (Posner & Plum, 1960; Vorstrup *et al.* 1984). However, no studies have examined the acute effects of a single dose of ACZ on CBF, cerebrovascular CO_2 reactivity and breathing stability at both sea level and at high altitude. Therefore, although plausible, it remains unclear whether acute I.V. ACZ administration at high altitude alters cerebrovascular function and subsequently modulates breathing stability.

In the present study, we examined the effect of I.V. ACZ on cerebrovascular and ventilatory responsiveness to CO_2 and O_2 at sea level and following partial acclimatisation to 5050 m. We selected to use the I.V. route of administration to avoid the confounding factor of altered acid–base balance associated with oral ACZ administration (Swenson, 1998). Based on previous studies that show a worsening of breathing stability following experimental reductions in CBF and cerebrovascular CO_2 reactivity (Xie *et al.* 2009; Fan *et al.* 2010*b*), we tested the hypothesis that acute ACZ-induced elevations in CBF and reactivity would lead to stabilisation of breathing control in partially acclimatised newcomers to 5050 m. These experiments were part of a series investigating the cardiorespiratory and cerebrovascular adaptations and mechanisms of regulation over 14 days at 5050 m. Although some of the experiments performed during this expedition have already been published (Fan *et al.* 2010*a*,*b*; Thomas *et al.* 2010; Lucas *et al.* 2011), apart from the experimental design and control data at high altitude from half of this group (Lucas *et al.* 2011), there is no overlap or duplication of data with the present study described herein.

Methods

Participants

Twelve sea-level residents (eight male and four female) with a mean age of 30 ± 10 years (mean ± SD) and body

mass index of 23 ± 2 kg m^{-2} participated in this study. Participants were non-smokers, had no previous history of cardiovascular, cerebrovascular or respiratory diseases and were not taking any medication.

Ethical approval

The study was approved by the Lower South Regional Ethics Committee of Otago and conformed to the standards set by the *Declaration of Helsinki*. All participants were informed regarding the purposes and procedures of this study, and informed consent was given prior to participation.

Experimental design

After a full familiarisation with the experimental procedures outlined below (on the first visit), the participants underwent experimental trials at sea level and at 5050 m (the Ev-K2-CNR Pyramid Laboratory, Nepal; barometric pressure 413 ± 1 mmHg). At sea level, participants underwent three experimental trials in randomised order (ACZ, indomethacin and placebo). Due to time constraints, no placebo trial was carried out at 5050 m. The ascent profile from sea level (Dunedin, New Zealand) to the Pyramid Laboratory has been described previously (Fan *et al.* 2010*a*). To avoid any confounding influence of acute mountain sickness (AMS), experimental sessions were carried out between days 5–11 (7 ± 2 days) after arrival to 5050 m, after any symptoms from AMS had subsided. The present study was part of a larger experiment – to be reported elsewhere – in which the participants were administrated indomethacin (100 mg, oral) on a separate day (single-blinded, randomised order and separated by at least 3 days). Before each experimental session, participants abstained from exercise and alcohol for 24 h, caffeine for 12 h and a heavy meal for 4 h prior.

With the exception of the arterial blood gas sampling, which was conducted following a 10 min supine rest, all experiments were performed with participants in a semi-recumbent position. Following 10–15 min of quiet rest, each experimental testing session consisted of: (a) an arterial blood gas sample; (b) instrumentation; (c) 5 min resting baseline; (d) modified hyperoxic rebreathing and isocapnic hypoxia (see details of methods below); (e) ACZ administration (I.V. 10 mg kg^{-1}) or placebo (sea level only); (f) 30 min rest; and (g) repeat testing of a–d. The order of the modified rebreathing and isocapnic hypoxic rebreathing was randomised and 5 min recovery was permitted between each trial to restore end-tidal gases to baseline resting values.

Ventilatory stability

Details of the ventilatory stability analysis have been previously described (Fan *et al.* 2010*b*). In brief, we first established whether unstable breathing patterns were present by visually analysing the ventilation ($\dot{V}_E$) traces obtained during spontaneous room air breathing during the final 2 min of the 5 min baseline period, based on absence or presence of Cheyne–Stokes-like respiratory patterns, characterised by distinct waxing and waning of tidal volume (V_T) and breathing frequency (f) pattern (Cherniack & Longobardo, 1973). If the trace was considered unstable, the difference between the peak and trough of any oscillation in $\dot{V}_E$ was measured. The average of this $\dot{V}_E$ difference (magnitude of $\dot{V}_E$ oscillation) was subsequently used as an index of ventilatory stability. The number of oscillations during this 2 min period was also noted. In addition, we calculated the co-efficient of variation of the breath-by-breath $\dot{V}_E$, V_T and f data (variability) during the 2 min period.

Modified rebreathing method

The modified rebreathing method is a well-established method for assessing both ventilatory and cerebrovascular CO_2 reactivities (Ainslie & Duffin, 2009). We selected the modified rebreathing method over a steady-state method to limit the confounding influence of a P_{CO_2} gradient on ventilatory control (Fan *et al.* 2010*b*). Since hyperoxia ($P_{a,O_2} \geq 150$ mmHg) diminishes peripheral chemoreceptors' output (Cunningham *et al.* 1963; Gardner, 1980), the ventilatory response to the modified rebreathing method can be interpreted as the ventilatory CO_2 sensitivity primarily from the central chemoreflex. The details of the modified rebreathing method have been previously described in Fan *et al.* (2010*a*). In brief, the participants wore a nose clip and breathed through a mouthpiece connected to a Y-valve which allowed switching from room air to a 6 l rebreathing bag filled with 7% CO_2 and 93% O_2. Following baseline room air breathing, participants were instructed to hyperventilate for 5 min to lower and then maintain a partial pressure of CO_2 (P_{ET,CO_2}) at 22 ± 2 mmHg (sea level) or 17 ± 3 mmHg (5050 m). Participants were then switched to the rebreathing bag following an expiration and instructed to take three deep breaths to ensure rapid equalisation of P_{CO_2} in the rebreathing circuit. The rebreathing tests were terminated when either: (i) P_{ET,CO_2} reached 60 mmHg; (ii) partial pressure of end-tidal O_2 (P_{ET,O_2}) dropped below 160 mmHg; (iii) $\dot{V}_E$ exceeded 100 l min^{-1}; or (iv) the participant reached the end of their tolerance.

The rebreathing data were analysed on a breath-by-breath basis using a specially designed programme (Full Fit Rebreathing programme, Version 3.1, University of Toronto, Toronto, Canada) and the analysis has been described in detail previously (Mohan *et al.* 1999; Duffin *et al.* 2000).

Isocapnic hypoxia

The soda-lime rebreathing technique was used to assess the ventilatory O_2 sensitivity as an index of peripheral chemoreflex sensitivity (Mathew *et al.* 1983). Participants wore a nose clip and breathed through a mouthpiece connected to a Y-valve allowing switching from room air to a circuit consisting of a 6 l rebreathing bag and a soda-lime reservoir. The protocol began with baseline room air breathing, before participants were switched to the rebreathing circuit at the end of an inspiration. Participants filled the rebreathing bag with room air drawn in through their nose and expired into the bag. Once the bag was filled (ensuring that this was at the end of expiration) the nose clip was attached and rebreathing began. The isocapnic hypoxia was terminated when either: (i) peripheral O_2 saturation (S_{p,O_2}) reached 80% at sea level and 70% at 5050 m; (ii) P_{ET,O_2} decreased to 45 mmHg at sea level and 30 mmHg at 5050 m; (iii) the $\dot{V}_E$ exceeded 100 l min^{-1}; or (iv) the participant reached the end of their tolerance.

The breath-by-breath $\dot{V}_E$ data during the isocapnic hypoxic rebreathing were plotted against P_{ET,O_2} and an inverse first-order polynomial function was used to obtain the hypoxic ventilatory response curve (Day & Wilson, 2009):

$$y = y_0 + \frac{c}{x}$$

where y_0 is the y asymptote, x is the P_{ET,O_2} in mmHg and c is the curvature (representing the responsiveness).

Steady-state hypocapnia

Voluntary hyperventilation was used to assess cerebrovascular reactivity to hypocapnia (Xie *et al.* 2005). Steady-state hypocapnic cerebrovascular reactivity was estimated from the slope of the reduction in mean MCAv from the final 2 min of baseline to the voluntary hyperventilation which preceded rebreathing, relative to the reduction in P_{ET,CO_2} (see below). We did not control for F_{I,O_2} during the voluntary hyperventilation because the small increases in P_{ET,O_2} (by 29 ± 11 and 16 ± 12 mmHg at sea level and 5050 m, respectively) were considered unlikely to influence either MCAv or its reactivity.

ACZ administration

Slow (~60 s) I.V. administration of ACZ (10 mg kg^{-1}; Diamox) or placebo (saline; sea level only) was achieved via an indwelling catheter located in an antecubital vein. The ACZ dose was reconstituted in approximately 5 ml of saline; a comparable volume (7–10 ml, depending on body mass) of I.V. saline was administered in the placebo trial. At sea level, the order was randomised and the participants were blinded to the condition.

Measurements

Respiratory variables. $\dot{V}_E$ and its components of V_T and f were measured using a heated pneumotachograph (Hans-Rudolph 3813) and expressed in units adjusted to BTPS. P_{ET,O_2} and P_{ET,CO_2} were measured using fast-responding gas analysers (model CD-3A, AEI Technologies, Pittsburgh, PA, USA; ML206 and ML240, ADInstruments, Colorado Springs, CO, USA). The pneumotachograph was calibrated using a 3 l syringe (Hans-Rudolph 5530) and the gas analysers were calibrated using gas mixtures of known concentrations of O_2 and CO_2 prior to each testing session.

Cerebrovascular variables. Cerebral blood flow velocity was measured in the right middle cerebral artery using a 2 MHz pulsed Doppler ultrasound system (MCAv; DWL, Compumedics Ltd, Germany). The Doppler ultrasound probe was positioned over the right temporal window and held in place with an adjustable headband. Optimal signals were obtained using search techniques described elsewhere (Aaslid *et al.* 1982). Beat-to-beat mean arterial blood pressure (MAP) was monitored using finger photoplethysmography (Finometer, Finapress Medical Systems, the Netherlands). Manual blood pressure measurements by auscultation were also made periodically to check and validate the automated recordings. Cerebrovascular conductance index (CVCi) was subsequently estimated by dividing mean MCAv by MAP within each breath cycle to reveal intrinsic vascular responses to CO_2.

Arterial blood gases. Arterial blood gas samples from a radial artery were obtained at rest using a 25-gauge needle into a pre-heparinised syringe. Following standardised calibration, all blood samples were analysed using an arterial blood-gas analysing system (NPT 7 series, Radiometer, Copenhagen, Denmark) for pH, partial pressure of arterial O_2 (P_{a,O_2}) and CO_2 (P_{a,CO_2}), bicarbonate concentration ($[HCO_3^-]$) and arterial O_2 saturation (S_{a,O_2}).

With the exception of the arterial blood gas variables, all data were acquired at 1000 Hz using an analog-to-digital converter (PowerLab; ADInstruments) with commercially available software (Chart version 5.5.6, ADInstruments), and stored on computer for later analysis.

Statistical analysis

The effects of altitude and ACZ on resting variables, cardiorespiratory and cerebrovascular responsiveness to CO_2, as well as the ventilatory variability were assessed

Table 1. Resting cerebrovascular, respiratory, cardiovascular and arterial blood gas variables before and after I.V. acetazolamide (ACZ) at sea level and following partial acclimatisation to 5050 m

	Sea level		5050 m	
	Control	ACZ	Control	ACZ
Cerebrovascular				
MCAv (cm s^{-1})	70 ± 13	80 ± 16†	73 ± 12	94 ± 15†
CVCi (cm s^{-1} $mmHg^{-1}$)	0.88 ± 0.21	0.95 ± 0.20	0.82 ± 0.17	1.08 ± 0.26†
Respiratory				
$\dot{V}_E$ (l min^{-1})[a]	14.3 ± 3.9[a]	13.0 ± 3.7†	17.4 ± 3.0[c]	16.6 ± 2.7*†[a]
f (breaths min^{-1})	15 ± 4[a]	14 ± 4†	18 ± 5*	17 ± 3*†
V_T (l)	0.95 ± 0.13[a]	0.95 ± 0.16	1.02 ± 0.31[c]	0.99 ± 0.22[a]
P_{ET,CO_2} (mmHg)	42 ± 5	40 ± 5†	24 ± 3*	25 ± 3*†
P_{ET,O_2} (mmHg)	103 ± 6	103 ± 5	47 ± 4*	49 ± 7*
Cardiovascular				
MAP (mmHg)	80 ± 10	84 ± 7	90 ± 9	89 ± 15
HR (beats min^{-1})	68 ± 9	60 ± 8†	77 ± 11*	77 ± 11*
Arterial blood gases				
pH	7.46 ± 0.04[b]	7.44 ± 0.04†[b]	7.46 ± 0.02	7.44 ± 0.02†
P_{a,CO_2} (mmHg)	42 ± 4[b]	42 ± 4[b]	27 ± 4*	31 ± 3*†
P_{a,O_2} (mmHg)	97 ± 6[b]	93 ± 8†[b]	46 ± 4*	42 ± 3*†
S_{a,O_2} (%)	98.1 ± 0.5[b]	97.5 ± 0.5†[b]	82.9 ± 3.3*	79.2 ± 2.7*†
$[HCO_3^-]$ (mmol l^{-1})	29.8 ± 3.0[b]	28.7 ± 3.1[b]	18.7 ± 3.0*	20.7 ± 2.7*†
SBE^-	6.1 ± 2.9[b]	4.7 ± 3.2[b]	−3.9 ± 4.0*	−2.8 ± 2.7*

Values are means ± SD. *Different from sea level ($P < 0.05$); †different from control ($P < 0.05$). $n = 12$, except for [a]$n = 11$, [b]$n = 9$ and [c]$n = 10$.

using two-way (altitude and drug) repeated-measures ANOVA with an α-level of 0.05 (SPSS version 17.0, SPSS, Chicago, IL, USA). Pair-wise comparisons (Bonferroni corrected) were performed to isolate the effect of altitude and ACZ on the dependent measures within participants. Data are reported as mean ± SD.

Results

All 12 participants were able to complete the whole experimental protocol. Comparison of $\dot{V}_E$-CO_2 sensitivity could be carried out in only 11 participants, both at sea level and at 5050 m, due to a poor ventilatory flow trace during hyperoxic rebreathing. Comparisons of MAP-CO_2 reactivity were conducted in 9 and 10 participants at sea level and 5050 m, respectively, due to poor BP traces in remaining participants.

Resting variables (Table 1)

Cerebrovascular variables. No differences were observed in either MCAv or CVCi following ∼7 days of living at 5050 m ($P = 0.489$ and 0.452 *vs.* sea level, respectively). The administration of ACZ increased MCAv at sea level and at high altitude, with greater increases at altitude following partial acclimatisation to 5050 m (interaction effect: $P = 0.007$). Specifically, ACZ administration at sea level elevated MCAv by 15 ± 15% ($P = 0.003$ *vs.* control) without increasing CVCi ($P = 0.325$), whereas ACZ administration at 5050 m increased MCAv by 28 ± 11% ($P < 0.001$ *vs.* control) and CVCi by 33 ± 23% ($P = 0.001$ *vs.* control; interaction effect: $P = 0.046$).

Respiratory variables. Residing at high altitude elevated both $\dot{V}_E$ and f ($P = 0.030$ and 0.009, respectively), whereas administration of ACZ lowered these variables ($P = 0.045$ and 0.030), but not by a different extent at sea level *vs.* high altitude (interactions: $P = 0.636$ and 0.895). The V_T was unaffected by altitude ($P = 0.509$) and ACZ administration ($P = 0.674$). High altitude lowered P_{ET,CO_2} ($P < 0.001$), whereas ACZ administration had a differential effect on P_{ET,CO_2} at high altitude compared with at sea level (interaction effect: $P = 0.010$). Specifically, P_{ET,CO_2} was lowered by ACZ at sea level ($P = 0.027$ *vs.* control) but elevated at 5050 m ($P = 0.02$). In contrast, ACZ administration did not alter P_{ET,O_2} at sea level or its reduction at high altitude (altitude, $P < 0.001$; drug, $P = 0.427$; interaction, $P = 0.572$).

Cardiovascular variables. High altitude tended to elevate MAP ($P = 0.057$), whereas no effect of ACZ on MAP was evident for pooled altitudes ($P = 0.654$) or differentially between altitudes (interaction: $P = 0.294$). Resting HR was

Table 2. Effect of acetazolamide (ACZ) on breathing stability at sea level and following ascent to 5050 m

	Sea level		5050 m	
	Control	ACZ	Control	ACZ
Magnitude of $\dot{V}_E$ oscillation (Δ l min^{-1})	5.8 ± 5.8	2.5 ± 5.3†	7.7 ± 5.2	5.9 ± 6.1†
Frequency of $\dot{V}_E$ oscillation (event min^{-1})	1.3 ± 1.2	0.3 ± 0.6†	3.0 ± 0.5*	1.8 ± 0.4*†
Variability				
$\dot{V}_E$ (%)	29 ± 17[a]	29 ± 17†[a]	36 ± 17[a]	22 ± 10†[a]
f (%)	18 ± 5[a]	16 ± 8†[a]	23 ± 10[a]	16 ± 7†[a]
V_T (%)	26 ± 14[a]	27 ± 19[a]	37 ± 17[a]	21 ± 10†[a]

Values are means ± SD. *Different from sea level ($P < 0.05$); †different from control ($P < 0.05$). n = 12, except for a n = 11.

elevated at high altitude ($P = 0.017$). The administration of ACZ lowered resting HR at sea level ($P = 0.002$ *vs.* control) but not at 5050 m ($P = 0.650$; interaction effect: $P = 0.021$).

Arterial blood gas variables. At 5050 m, pH was similar to sea-level values ($P = 0.700$), indicating complete renal correction of the hypoxia-induced respiratory alkalosis and therefore well-advanced acclimatisation to this altitude at this time point. The P_{a,O_2} and SBE$^-$ both remained lower than at sea level ($P = 0.001$). Regardless of the altitude, ACZ administration lowered pH and P_{a,O_2}, while SBE-remained unchanged (drug effects: $P = 0.015$, 0.012 and 0.562, respectively; interaction effects: $P = 0.831$, 0.936 and 0.134). Ascent to 5050 m lowered resting P_{a,CO_2}, S_{a,O_2} and [HCO_3^-] (all $P < 0.001$ *vs.* sea level). The effect of ACZ on P_{a,CO_2}, S_{a,O_2} and [HCO_3^-] was greater at 5050 m compared with at sea level (interaction effects: $P = 0.003$, 0.018 and 0.024). Specifically, ACZ at sea level lowered S_{a,O_2} ($P = 0.013$) without measurably changing P_{a,CO_2} or [HCO_3^-] ($P = 1.000$ and 0.342), but at high altitude it caused a larger reduction in S_{a,O_2} ($P = 0.002$) with elevations in resting P_{a,CO_2} and [HCO_3^-] (both $P < 0.001$).

Following the placebo trial at sea level, cardiorespiratory, cerebrovascular and blood gas variables were unchanged (data not shown, for clarity).

Breathing stability (Table 2)

Ascent to 5050 m increased the frequency of $\dot{V}_E$ oscillation ($P < 0.001$; interaction effect, $P = 0.551$), but did not alter its magnitude, or the variability of f, V_T or $\dot{V}_E$ ($P = 0.172$, 0.249, 0.076 and 0.929). At sea level and 5050 m, ACZ administration reduced both the magnitude and frequency of the $\dot{V}_E$ oscillations ($P = 0.027$ and < 0.001 *vs.* control, respectively; interaction effect, $P = 0.489$ and 0.551); the variability in f and $\dot{V}_E$ were also reduced ($P = 0.033$ and 0.014, respectively; interaction effect, $P = 0.283$ and 0.067). The ACZ administration reduced the variability of V_T at 5050 m ($P = 0.011$) but not at sea level ($P = 0.615$; interaction effect, $P = 0.042$).

Modified rebreathing

Cerebrovascular CO_2 reactivity (Fig. 1). Ascent to 5050 m elevated the rebreathing hypercapnic MCAv-CO_2 reactivity by 114 ± 146% ($P = 0.008$ *vs.* sea level). ACZ administration elevated hypercapnic MCAv-CO_2 reactivity ($P = 0.030$) both at sea level and 5050 m (interaction effect, $P = 0.355$). One participant displayed a high cerebrovascular reactivity to hypercapnic following ACZ at sea level. Re-analysis of the data with this individual excluded revealed a tendency for the hypercapnic MCAv-CO_2 to be elevated with ACZ at sea level ($P = 0.052$). Ascent to 5050 m elevated the hypocapnic MCAv-CO_2 reactivity by 114 ± 71% ($P < 0.001$ *vs.* sea level). The administration of ACZ elevated hypocapnic MCAv-CO_2 reactivity at sea level (by 31 ± 43%; $P = 0.026$) and by a greater amount at 5050 m (by 69 ± 60%; $P < 0.001$; interaction effect, $P = 0.003$).

Ventilatory CO_2 sensitivity (Fig. 2). High altitude tended to elevate the $\dot{V}_E$-CO_2 sensitivity ($P = 0.085$). Meanwhile, no changes were observed in the $\dot{V}_E$-CO_2 sensitivity following ACZ administration ($P = 0.883$), irrespective of the altitude (interaction, $P = 0.127$).

Cardiovascular CO_2 reactivity (Fig. 2). Ascent to 5050 m elevated both MAP-CO_2 reactivity and HR-CO_2 reactivity ($P = 0.003$ and 0.006 *vs.* sea level, respectively). In contrast, these reactivities were unchanged following ACZ administration ($P = 0.866$ and 0.611), irrespective of the altitude (interaction, $P = 0.105$ and 0.475, respectively).

Isocapnic hypoxia

Ascent to 5050 m elevated the $\dot{V}_E$-O_2 sensitivity by (2420 ± 1521 *vs.* 1020 ± 908 units; $P = 0.002$ *vs.* sea level). No changes were observed in the $\dot{V}_E$-O_2 sensitivity with ACZ administration ($P = 0.559$ *vs.* control) at either sea level (981 ± 1152 *vs.* 1057 ± 563 units) or following ascent to 5050 m (2358 ± 1496 *vs.* 2482 ± 1616 units; interaction effect, $P = 0.958$, data not shown).

Discussion

We have examined the acute effects of a single I.V. dose of ACZ on cerebrovascular and ventilatory responsiveness to CO_2 and hypoxia in healthy resting individuals at sea level and following partial acclimatisation to 5050 m. The main novel findings were that ACZ at 5050 m: (1) elevated resting MCAv (to a greater extent than at sea level) and P_{a,CO_2}, while it lowered both S_{a,O_2} and P_{a,O_2}; (2) improved breathing stability as reflected in a reduced variability of $\dot{V}_E$, V_T and incidence of breathing oscillations, while the ventilatory responsiveness to hypercapnia and hypoxia were unchanged; and (3) elevated both hypercapnic and hypocapnic cerebrovascular CO_2 reactivity, with the hypocapnia reactivity increases greater than observed at sea level. Our data thus indicate that ACZ administration at 5050 m improves breathing stability during wakefulness without concurrent improvements in arterial O_2 saturation or P_{a,O_2}. Moreover, the improvement in breathing patterns following ACZ occurred independently of any measurable changes in the sensitivity of the central or peripheral chemoreflexes. We therefore speculate that P_{a,CO_2}-mediated elevations in cerebral perfusion and an enhanced cerebrovascular reactivity may partly account for the improved breathing stability following ACZ at high altitude

Limitations

An important limitation of the present study is the assumption that MCAv represents global CBF changes. Subudhi *et al.* (2011) pointed out that since CBF is markedly heterogenous during hypoxia, ACZ may have

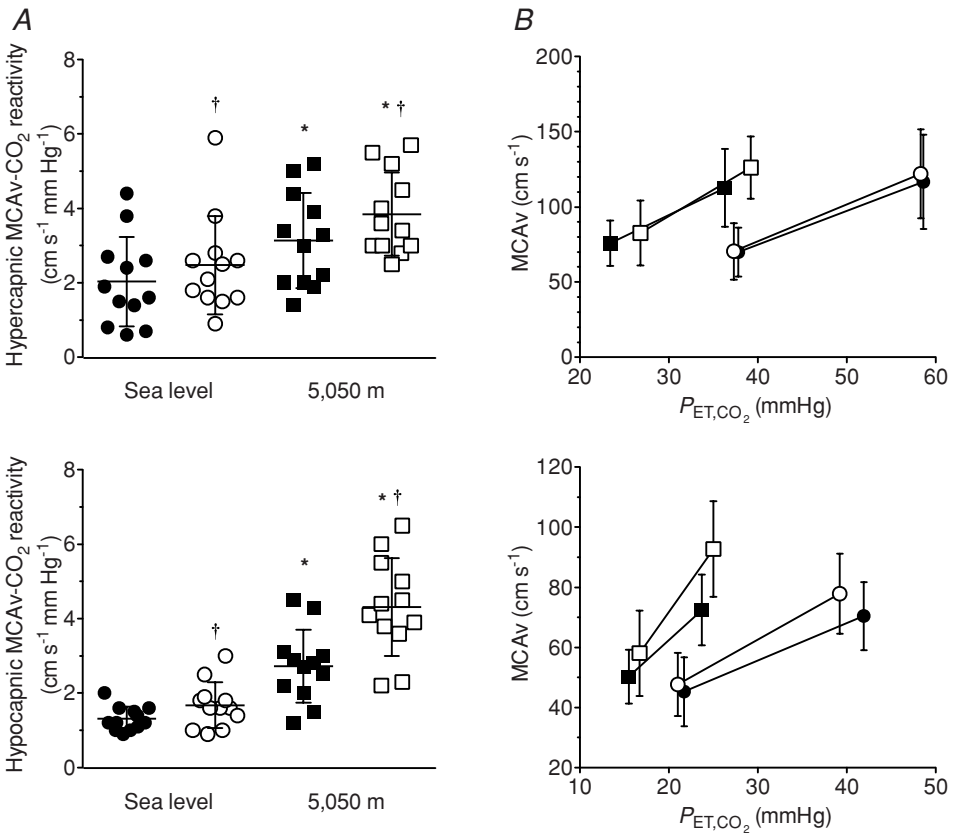

Figure 1. Effects of acetazolamide (ACZ) and high altitude (5050 m) on cerebrovascular responsivess to hypercapnia and hypocapnia
A, individual slopes; *B*, group data (mean ± SD). Filled circles, sea level control; open circles, sea level ACZ; filled squares, 5050 m control; open squares, 5050 m ACZ. ACZ elevated the hypercapnic and the hypocapnic cerebrovascular reactivity at both sea level and at 5050 m. *Different from sea level ($P < 0.05$); †different from control ($P < 0.05$).

regional specific effects not detected with transcranial Doppler. ACZ administration (10 mg kg^{-1}, I.V.) in rats uniformly elevates CBF to the frontal and parietal cerebral cortex, the striatum, the hippocampus and the cerebellum, reaching a maximum at 1 h and progressively returning to baseline over 6 h (LaManna & McCracken, 1990). Likewise, ACZ (1 g) elevated CBF in numerous cortical grey matter areas (occipital lobe, superior frontal gyrus, cingulate gyrus, primary sensory-motor cortex, middle and superior temporal gyrus) and putamen and white matter, which was quantified using both MRI and PET measurements in healthy humans (Grandin *et al.* 2005). In addition, a MR imaging study by Schreiber *et al.* (2000) reported no changes in MCA diameter with ACZ administration in patients with internal carotid occlusion. Finally, a recent report showed a preserved MCA diameter

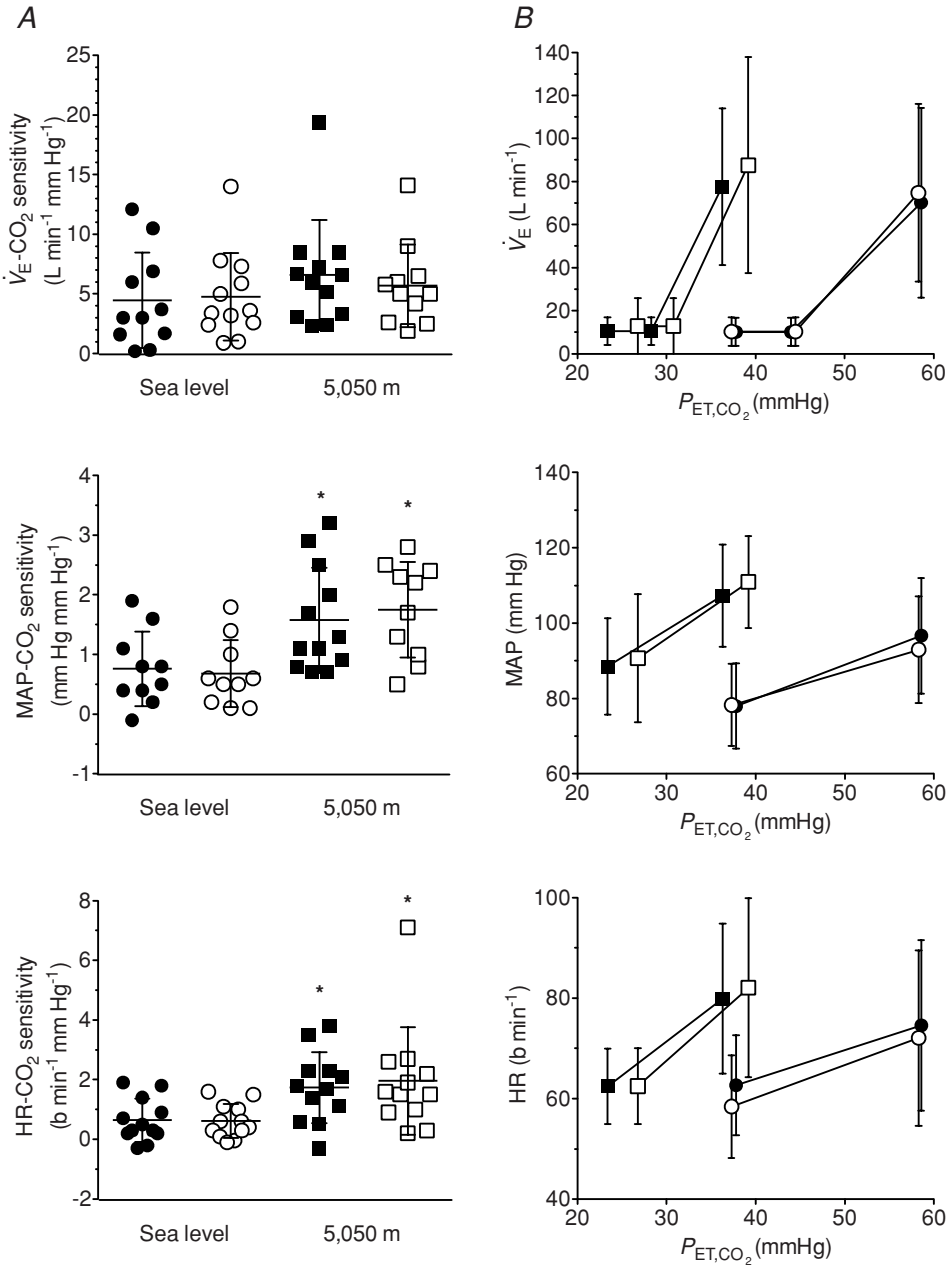

Figure 2. Effects of acetazolamide (ACZ) and high altitude (5050 m) on ventilatory and cardiovascular responsivess to hypercapnia
A, individual slopes; *B*, group data (mean ± SD). Filled circles, sea level control; open circles, sea level ACZ; filled squares, 5050 m control; open squares, 5050 m ACZ. Ascent to 5050 m enhanced the cardiovascular response to CO_2, while no changes were observed following ACZ at either sea level or 5050 m. *Different from sea level ($P < 0.05$).

at 5300 m compared with sea level (Wilson *et al.* 2011). Taken together, we contend that: (i) ACZ elevates CBF in all major brain regions; (ii) the ACZ-induced changes in MCAv reflect these global changes in CBF; and (iii) MCA diameter is unlikely to be altered at 5050 m.

We selected to use the I.V. route rather than oral administrations to examine the influence of the acute direct effect of ACZ on CBF without the major confounding influence of changes in acid–base balance following oral administration (Hauge *et al.* 1983; Vorstrup *et al.* 1989; see Swenson, 1998 for review). Nevertheless, upon injection of ACZ, an unexpected observation was the apparent changes in pH and P_{a,CO_2} at 5050 m (Table 1). Carbonic anhydrase inhibition causes disequilibrium of the CO_2 buffer system, which results from an incomplete hydration of CO_2 during the passage of circulating blood through the vascular beds. There is a progressive elevation of arterial CO_2 tension from the lungs to the peripheral tissues (Brzezinski *et al.* 1967). In the present study, there were several minutes of delay between the arterial blood sampling and analysis, thus allowing sufficient time for the equilibrium to complete. Therefore, it is likely that the true *in vivo* $[H^+]$ and P_{a,CO_2} values might be overestimated by our arterial blood gas measurements following ACZ. Nevertheless, Brzezinski *et al.* (1967) found a consistent relationship between P_{a,CO_2} with direct cerebral tissue CO_2 tension (~6 mmHg difference) before and following ACZ in anaesthetised dogs. Accordingly, we believe that the arterial blood gas values obtained in the present study provide an insight to the differential effect of ACZ on acid–base balance at 5050 m. Our findings of a greater elevation in MCAv at 5050 m following ACZ is probably explained by the greater ACZ-induced elevations in P_{a,CO_2} (Table 1).

Acetazolamide elevates cerebral blood flow at high altitude

While the influence of ACZ on CBF has been studied extensively during normoxia (Posner & Plum, 1960; Ehrenreich *et al.* 1961; Hauge *et al.* 1983; Vorstrup *et al.* 1984), its effect on cerebrovascular function during hypoxic exposure is poorly understood and entirely based on oral administration of the drug. For example, studies have found either reduced (Subudhi *et al.* 2011) or preserved (Teppema *et al.* 2007) CBF velocity in response to short-duration hypoxia (4–8 h) following 1 and 3 day oral ingestion of ACZ, respectively (250 mg every 8 h). At 3475 m, Jensen *et al.* (1990) reported a 22% increase in CBF velocity 2 h following oral ACZ ingestion (1.5 g). Vuyk *et al.* (2006) found higher cerebral oxygenation during exercise at 3700 m and with ACZ (750 mg daily) compared with untreated controls. In the present study, acute ACZ administration at 5050 m increased resting MCAv by 28% and elevated the hypercapnic and hypocapnic MCAv-CO_2 reactivity by 51% and 69%, respectively (Table 1 and Fig. 1). Our data thereby demonstrate, for the first time, that acute I.V. ACZ administration elevates CBF and enhances the cerebrovascular response to changes in CO_2 in partially acclimatised newcomers to 5050 m. Such elevation in resting CBF with ACZ at 5050 m would also serve to improve cerebral oxygen delivery and thus potentially attenuate cerebral hypoxaemia.

Acetazolamide exacerbates hypoxaemia at 5050 m

Previous studies have found either unchanged (Teppema *et al.* 2006, 2007, 2010) or improved (Schoene *et al.* 1983; Burki *et al.* 1992; Mirrakhlmov *et al.* 1993; Subudhi *et al.* 2011) arterial O_2 saturation with oral ACZ – presumably related to the changes in resting $\dot{V}_E$ associated with carbonic anhydrase inhibition. In the present study, we observed a slight reduction in both P_{a,O_2} and S_{a,O_2} following ACZ at both sea level and following ascent to 5050 m (Table 1). We attributed this increase in arterial hypoxaemia to hypoventilation (see below) and/or the Bohr effect, whereby ACZ-induced CO_2 retention causes less O_2 loading of the red blood cells in the lungs.

Carbonic anhydrase inhibition and breathing control

Despite the large body of literature (see Swenson, 1998 for review), much controversy still surrounds the effects of ACZ-induced carbonic anhydrase inhibition on ventilatory control. The effect of carbonic anhydrase inhibition on ventilatory control depends critically on the dose and the route of ACZ administration (Teppema *et al.* 2001). For example, oral administration of ACZ usually results in metabolic acidosis, which is frequently (but not always) associated with a rise in $\dot{V}_E$ (Teppema & Dahan, 1999). Meanwhile, I.V. ACZ administration does not immediately lead to metabolic acidosis, but may be followed by inhibition of ventilatory control (Swenson, 1998).

One important factor of carbonic anhydrase inhibition on resting $\dot{V}_E$ is the effect of elevated CBF and associated brain CO_2 washout. Hauge *et al.* (1983) proposed that, assuming constant cerebral CO_2 production, an increase in resting CBF and its responses to CO_2 associated with cerebral capillary carbonic anhydrase inhibition would lower brain tissue P_{CO_2} via an increase of CO_2 washout, thus attenuating central chemoreceptor activation. In support of this, they observed an initial depression of $\dot{V}_E$ with carbonic anhydrase inhibition, which coincided with the ACZ-induced increase in resting CBF. In the present study, we also observed a reduction in resting $\dot{V}_E$ following ACZ at both sea level and 5050 m (Table 1). Our finding contradicts previous reports of elevated resting $\dot{V}_E$

following chronic oral ACZ administration (Swenson & Hughes, 1993; Teppema & Dahan, 1999; Teppema *et al.* 2007, 2010). Since we observed a ∼15% increase in resting MCAv at 30 min following acute ACZ administration at sea level, while arterial pH was lowered (Table 1), we speculate that the reduction in resting $\dot{V}_E$ may be due to the effect of increased CBF and associated central H^+ washout, overriding any stimulating effect of ACZ-induced CO_2 retention (Coates *et al.* 1991; Teppema *et al.* 1995, 2010). Accordingly, it appears that the ventilatory effect observed with ACZ may be closely linked with the changes in the control of CBF.

In the present study, both $\dot{V}_E$-CO_2 and $\dot{V}_E$-O_2 sensitivities remained unchanged with acute ACZ administration (10 mg kg^{-1}, I.V.) at both sea level and 5050 m (Fig. 2). However, in agreement with previous findings (Teppema *et al.* 1992, 2006; Swenson & Hughes, 1993; Teppema & Dahan, 2004), we did observe complete abolishment of the ventilatory response to hypoxia in some participants at both sea level ($n = 3$) and 5050 m ($n = 1$) with ACZ. In addition, there was a rightward shift of the $\dot{V}_E$-CO_2 slope during modified rebreathing with ACZ at 5050 m (Fig. 2). Our finding is in contrast to previous reports of a leftward shift of the $\dot{V}_E$-CO_2 slope with ACZ (Teppema & Dahan, 1999). However, since that study assessed the ventilatory CO_2 sensitivity following 3 days of oral ACZ ingestion (250 mg 8 h^{-1}), differences in the method of administration (oral *vs.* I.V.), drug dosage and assessment of ventilatory response (end-tidal forcing *vs.* rebreathing) probably account for these discrepant findings.

Acetazolamide and breathing instability

In the present study, we found acute ACZ administration at 5050 m improved breathing stability (Table 2), despite preserved $\dot{V}_E$-CO_2 and $\dot{V}_E$-O_2 sensitivities (Fig. 2). Our data indicate that this improved breathing stability is mediated by reductions in variability of the tidal breath volumes and the frequency of breathing oscillations (i.e. Cheyne–Stokes respiration, Table 2). Our findings provide support to the notion that ACZ ameliorates breathing pattern disturbances following ascent to high altitude (Weil *et al.* 1978; Sutton *et al.* 1979, 1980; Hackett *et al.* 1987), independent of changes in either peripheral (Hackett *et al.* 1987) or central chemoreflexes (Burki *et al.* 1992). Furthermore, our findings corroborate with those of Gotoh *et al.* (1969) who found that I.V. ACZ injection (500 mg) improved breathing stability by reducing the incidence of Cheyne–Stokes respiration cycles in patients with cerebrovascular disease. We partly attribute the improvement in breathing stability with ACZ to the elevations in CBF velocity and related elevations in cerebrovascular responsiveness to changes in CO_2 (Fig. 1). Indeed, it has been shown that reduction in resting CBF and cerebrovascular hypocapnic reactivity lowers the CO_2 reserve (the difference between eupnoeic P_{ET,CO_2} and apnoea threshold) and increases the risk of unstable breathing during sleep (Xie *et al.* 2009). Xie and colleagues attributed this reduction in CO_2 reserve to an increased slope of ventilatory response to CO_2 *below* eupnoea (i.e. ventilatory controller gain) associated with *reduced* cerebrovascular hypocapnic reactivity. As such, it seems reasonable that increases in CBF and cerebrovascular hypocapnic reactivity associated with ACZ would therefore blunt the ventilatory slope to CO_2 below eupnoea, thereby increasing the CO_2 reserve and improve breathing stability. Moreover, since hypoventilation lowers the CO_2 reserve (Dempsey, 2005), we speculate that the stabilising effect of ACZ must be greater than the destabilising effect of the reduced ventilation observed in the present study (Table 1). In support of this, ACZ has been found to increase the CO_2 reserve by lowering the apnoeic threshold in humans (Teppema *et al.* 2010) and in the anaesthetised cat models (Teppema *et al.* 2001). This improvement in the CO_2 reserve would account for the therapeutic effect of ACZ on breathing stability at high altitude. Together with data from the present study, these findings indicate that ACZ-induced changes in cerebrovascular function serve to modulate ventilatory controller gain *below* eupnoea, and thus increase the CO_2 reserve and improve breathing stability at 5050 m. However, we cannot exclude the possibility that ACZ may improve breathing stability via alterations in the peripheral chemoreceptor response, which was not detected in the present study, since carbonic anhydrase inhibition is known to (i) increase carotid body sinus activity delay (Lahiri *et al.* 1982; Iturriaga *et al.* 1991), (ii) lower carotid body activity in response to steady state CO_2 *in vivo* (Hayes *et al.* 1976; Lahiri *et al.* 1976), and (iii) attenuate the speed of response to CO_2 *in vitro* (Iturriaga *et al.* 1991). Nevertheless, the increased cerebrovascular hypocapnic reactivity with ACZ may also account for the reduction in the incidence of central sleep apnoea in patients with congestive heart failure at sea level (Javaheri, 2006; Fontana *et al.* 2011). Indeed, Xie *et al.* (2005) previously reported a lower overall cerebrovascular CO_2 reactivity, especially to hypocapnia, in congestive heart failure patients with central sleep apnoea compared with patients without. Collectively, along with the related elevations in P_{a,CO_2}, it appears that changes in the hypocapnic cerebrovascular CO_2 reactivity may alter the CO_2 reserve, thus influencing breathing stability during both wakefulness and sleep. The potential implications for the role of altered cerebrovascular reactivity in the pathogenesis of breathing instability certainly warrant further investigation.

Conclusions

To our knowledge, the present study is the first to examine the effects of a single I.V. dose of ACZ on cerebrovascular function and breathing control in partially acclimatised newcomers to high altitude. We demonstrated that acute ACZ at 5050 m increased resting CBF (probably via elevations in P_{a,CO_2}) and enhanced the CBF responsiveness to both hypercapnia and hypocapnia, and improved breathing stability. We speculate that such elevations in CBF and cerebrovascular reactivity may account, in part, for the well-documented reductions in periodic breathing during sleep following oral ACZ ingestion at high altitude.

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Author contributions

P.N.A. and K.R.B. contributed to the conception and design of the experiment, the interpretation of the data and the writing of the manuscript. J.-L.F. carried out data collection, and led the analysis, interpretation and writing of the manuscript. K.C.P., K.N.T. and S.J.E.L. contributed equally in the study design and collection of data. J.D.C. and B.K. contributed to the data interpretation and manuscript preparation. All authors approved the final version of this manuscript. The sea-level work was carried out in the Human Physiology Laboratory, Department of Physiology, University of Otago, while the experiments at high altitude were carried out at the Pyramid Laboratory, Italian Research Council.

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