Influence of Cerebral Blood Flow on Central Sleep Apnea at High Altitude

Keith R. Burgess, PhD[1,2]; Samuel J.E. Lucas, PhD[3]; Kelly Shepherd, PhD[1]; Andrew Dawson, BSc[1]; Marianne Swart, MSc[1]; Kate N. Thomas, BSc[3]; Rebekah A.I. Lucas, PhD[3]; Joseph Donnelly, MBBS[3]; Karen C. Peebles, PhD[3]; Rishi Basnyat, MD[4,5]; Philip N. Ainslie, PhD[6]

[1]Peninsula Sleep Laboratory, Sydney, New South Wales, Australia; [2]Department of Medicine, University of Sydney, Sydney, New South Wales, Australia; [3]University of Otago, Dunedin, New Zealand; [4]Nepal International Clinic, Kathmandu, Nepal; [5]Banner Good Samaritan Medical Center, Phoenix, Arizona; [6]Centre for Heart, Lung and Vascular Health, School of Health and Exercise Sciences, University of British Columbia, Okanagan Campus, Canada

Study Objectives: To further our understanding of central sleep apnea (CSA) at high altitude during acclimatization, we tested the hypothesis that pharmacologically altering cerebral blood flow (CBF) would alter the severity of CSA at high altitude.

Design: The study was a randomized, placebo-controlled single-blind study.

Setting: A field study at 5,050 m in Nepal.

Patients or Participants: We studied 12 normal volunteers.

Interventions: Between days 5 to10 at high altitude, CBF velocity (CBFv) was increased by intravenous (IV) acetazolamide (10 mg/kg) and reduced by oral indomethacin (100 mg).

Measurements and Results: Arterial blood gases, hypoxic and hypercapnic ventilatory responses, and CBFv and its reactivity to carbon dioxide were measured awake. Overnight polysomnography was performed. The central apnea-hypopnea index was elevated following administration of indomethacin (89.2 ± 43.7 to 112.5 ± 32.9 events/h; mean ± standard deviation; $P < 0.05$) and was reduced following IV acetazolamide (89.2 ± 43.7 to 47.1 ± 48.1 events/h; $P < 0.001$). Intravenous acetazolamide elevated CBFv at high altitude by 28% (95% confidence interval [CI]: 22-34%) but did not affect ventilatory responses. The elevation in CBFv was partly mediated via a selective rise in partial pressure of arterial carbon dioxide ($PaCO_2$) (28 ± 4 to 31 ± 3 mm Hg) and an associated fall in pH ($P < 0.01$). Oral indomethacin reduced CBFv by 23% (95% CI: 16-30%), blunted CBFv reactivity, and increased the hypercapnic ventilatory response by 66% (95% CI: 30-102%) but had no effect on $PaCO_2$ or pH.

Conclusion: Our findings indicate an important role for cerebral blood flow regulation in the pathophysiology of central sleep apnea at high altitude.

Keywords: central sleep apnea, cerebral blood flow, cerebral vascular reactivity, high altitude, ventilatory responses

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INTRODUCTION

Although central sleep apnea (CSA) is not as common as obstructive sleep apnea (OSA), it remains a substantial and increasing clinical problem.[1] It occurs predominantly in patients with severe heart failure and some patients with stroke, and in normal people at high altitude. Existing treatments for CSA are suboptimal. Oxygen is commonly used in the treatment of CSA[2]; however, the results are variable, and although some investigators have shown benefit from continuous positive airway pressure (CPAP) therapy, the large Canadian trial of CPAP in chronic heart failure yielded a negative result.[3] Acetazolamide (ACZ) administered orally has been used experimentally in low doses, in limited numbers of patients,[4] but has not been accepted into mainstream practice, presumably because of side effects[5] and relative ineffectiveness. Carbon dioxide (CO_2) therapy has been shown to reduce the severity of CSA when administered constantly in a normal volunteer model[6] and more potently in a mathematical model when administered dynamically.[7] However, neither approach has been adopted into clinical practice. The pathogenesis of CSA remains incompletely understood, thus a better understanding of the underlying mechanisms/pathogenesis of CSA may provide improved treatment options.

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Address correspondence to: Keith R. Burgess, PhD, University of Sydney, Sydney, New South Wales, Australia; Tel: 61 2 9976 9548; Fax: 61 2 9976 9595; E-mail: keith.burgess@health.nsw.gov.au

Following ascent to high altitude by otherwise healthy individuals, CSA during sleep is almost universal, occurring in > 90% of people at an altitude above 5,000 m.[8] Experiments at high altitude provide insight into the pathogenetic mechanisms underpinning CSA, as well as potential therapeutic opportunities. The common trigger to both CSA in heart failure and high altitude exposure is transient reduction in the partial pressure of arterial carbon dioxide ($PaCO_2$)[9] below the apneic threshold.[10] The amount of decrease in $PaCO_2$ required will depend on the awake values, the ventilatory response to $PaCO_2$ below eupnea, and the position of the iso-metabolic line.[10,11] Other possible contributing factors, which have not been investigated extensively, especially following ascent to high altitude, are breathing pattern and cerebral blood flow (CBF), which are closely linked by the $PaCO_2$.[10,12] The effects of $PaCO_2$ on CBF provide an important protective mechanism that serves to minimize changes in the brain [H+], thereby stabilizing the breathing pattern in the face of perturbations in $PaCO_2$.[12,13] Hypocapnia normally causes marked cerebral vasoconstriction and reduces CBF, which attenuates the decrease in brain PCO_2 relative to that of the arterial blood. Accordingly, ventilatory inhibition in response to reduced brain PCO_2 will be lessened, because of the attenuated decrease in [H+] stimulus to central chemoreceptors. In addition, ascent to high altitude elevates sympathetic nerve activity and increases ventilatory responses to hypercapnia and hypoxia,[14] which will likely cause greater breathing instability because of increases in ventilatory "loop gain."[15] This has even greater significance during sleep, when $PaCO_2$ becomes critical in regulating the breathing pattern in the absence of the wakefulness drive to breathe.[16]

Table 1—Demographic features of subjects.

Subject No.	Age	Sex	BMI
1	25	M	24
2	26	F	23.4
3	29	F	19.7
4	22	M	21.8
5	24	F	23.1
6	31	M	24.7
7	56	M	20.7
8	42	M	26
9	33	M	21.7
10	20	M	20.1
11	28	M	25.2
12	23	F	23.4
Mean ± 1 SD	30 ± 10		22.8 ± 2.0

BMI, body mass index; 1 SD, 1 Standard Deviation.

It is known that CBF decreases at sleep onset in healthy individuals. In a previous study, in a small number of subjects, we found an association between the degree of reduction of CBF at sleep onset and the development of CSA during sleep at high altitude (3,900 m).[14] This observation generated our current hypothesis that changes in CBF will play an important role in the pathophysiology of CSA at high altitude. Although we clearly acknowledge the important role of the peripheral chemoreceptors, the main aim of this experiment was to test this hypothesis via the pharmacological manipulation of CBF in normal volunteers and assess its importance in the pathophysiology of CSA at high altitude.

METHODS

Twelve normal, healthy adults, (eight males and four females), with a mean age of 30 ± 10 y (mean ± standard deviation [SD]), and body mass index of 22.8 ± 2 kg/m^2 (Table 1), volunteered for this study, which was approved by the Lower South Regional Ethics Committee of Otago and the Nepal Health Medical Research Council and conformed to the standards set by the Declaration of Helsinki. Written informed consent was obtained. These experiments were planned for and conducted on the same expedition as those described in previous studies.[17,18] The evident relationships that the drug interventions have with sleep apnea severity are significant and the results are separate from those of the other experiments.

Experimental Design and Ascent Protocol

High-altitude exposure was chosen as a model for investigating the pathophysiology of CSA, because it is reproducible and relatively stable over at least 1 month, and can accommodate a large number of patients in and around a stable laboratory site over a period of several weeks.

All participants underwent full medical screening, including electrocardiography (ECG) and echocardiography assessment. Participants were not taking any medication, all were nonsmokers, 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.

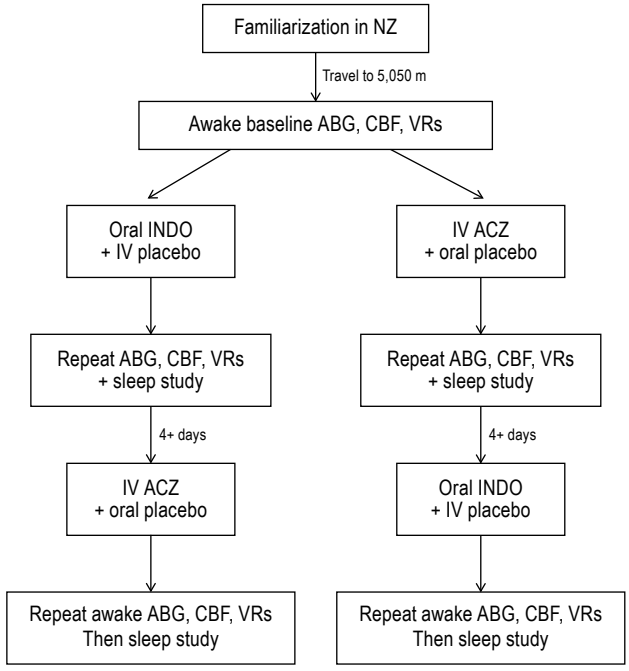

Figure 1—Overview of experimental design. NZ, New Zealand; IV, intravenous; ABG, arterial blood gas measurement; CBF, cerebral blood flow measurement; VRs, ventilatory response testing to hypoxia and hypercapnia; INDO, indomethacin; ACZ, acetazolamide.

Sea-level familiarization with the protocols was completed 2 weeks before arriving in Nepal. Participants spent 7 days at Kathmandu (~1,400 m) before flying to Lukla (2,860 m). Participants then trekked to the Ev-K2-CNR Pyramid Laboratory over an 8-day period, which included rest days at Namche Bazar (3,450 m) and Pheriche (4,252 m). During the first 7 days, all participants used a small dose of oral ACZ[5] during the trek to help speed acclimatization[19] and limit altitude illness. Importantly, treatment was discontinued > 48 h before reaching 5,050 m to allow sufficient clearance time. The reported half-life for oral acetazolamide is 10 hours and this low-dose quantity has been reported to be 90-100% excreted within 24 hours of administration[20]; therefore, it was unlikely to confound our findings. Furthermore, to avoid any confounding influence of Acute Mountain Sickness (AMS), experimental sessions were carried out between days 5-11 after arrival to 5,050 m (see Figure 1).

Pharmacological Manipulation of CBF

CBF was altered by the administration of licensed medications (oral indomethacin [INDO]) and intravenous [IV] ACZ)].[21] INDO, at a dose of 100 mg orally, reduces CBF velocity and its reactivity by 20-40% within 90 minutes, for up to 4 h.[21] IV ACZ can increase CBF by 20-50% within 30 minutes, for up to 8 hours. It has effects very different from oral ACZ. For example, when administered intravenously the effects are predominantly on CBF and extrarenal carbonic anhydrase, and it does not induce significant metabolic acidosis within this time. Using these two agents on different days, in a randomized

fashion (toss of coin for first drug allocation, then alternate allocation), we altered CBF in both directions, and examined the result of altering CBF on the severity of CSA and the potential underlying mechanisms (e.g., alterations in ventilatory responses and blood gases). INDO or placebo was administered orally approximately 90 minutes before testing began with 20 mL of an antacid solution, and ACZ or 0.9% saline was administered intravenously 30 minutes before testing began. The data were collected and analysed as "control," "drug 1" or "drug 2."

In addition, placebo controls were used to account for possible indirect effects of the medications. The placebo for INDO was an empty "INDO" gelatin capsule refilled with sugar, whereas normal saline was used as the IV ACZ placebo. Although different resting times were necessary between pre intervention and post intervention ventilatory response testing, only one participant (co-PI) knew this prior to data collection, and that person's data were not different from those of the rest of the group, who were intentionally blinded from this information.

Sleep Studies

All sleep studies were carried out with a Compumedics portable system (Somté PSG; Melbourne, Australia). Participants were set up for the polysomnogram by experienced polysomnography technologists according to standard format, as described in detail elsewhere.[8,22] All studies were scored by the same certified polysomnography technologist, who was blinded as to the nature of the study, using standard definitions.[23,24] The first 3 hours of sleep were used for analysis of the drug effects because the duration of action of INDO may be only 4 hours after onset, and the first hour was taken up with awake ventilatory response testing before sleep.

Experimental Procedures

With the exception of the arterial blood gas sampling, which was conducted following a 10-minute supine rest, all experiments were performed with participants in a semi-recumbent position. Following 10-15 minutes of quiet rest, each experimental testing session was composed of: (1) an arterial blood gas sample; (2) instrumentation; (3) 5-minute resting baseline; (4) steady-state hyperoxic hypercapnia, modified hyperoxic rebreathing, and isocapnic hypoxia (see details of methods in the following paragraphs); (5) drug intervention or placebo; (6) 30 or 90 minute rest; (7) repeat testing of 1-4; and (8) a night of full polysomnographic monitored sleep. Hyperoxic hypercapnia was intentionally used to eliminate the influence of hypoxic-induced peripheral chemoreceptor activation at high altitude and acutely remove the influence of hypoxia on cerebrovascular tone. The modified hypercapnia rebreathing protocol was preceded by a 5-minute period of voluntary hyperventilation, in accordance with the standardized protocol of Duffin.[25] The steady-state hyperoxic hypercapnic ventilatory response test was used only to determine the changes in cerebrovascular reactivity during 2 weeks of acclimatization to 5,050 m, reported elsewhere.[11] The order of the steady-state, modified rebreathing and isocapnic hypoxic rebreathing was randomized between participants, but was consistent within participants across all trials and before and after intervention, and full recovery (5 minutes) was permitted between each trial to restore end-tidal gases to baseline resting values. Because of equipment limitations, only four participants were studied each night. Therefore, it took 3 consecutive nights to study all 12 participants at each time point. All ventilatory testing was completed in the afternoon or early evening, and participants were instructed to avoid caffeine, alcohol, and exercise in the 12 hour prior to experimental testing.

Ventilatory Response Testing

Modified Hyperoxic Rebreathing Method

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% oxygen (O_2). Following baseline data collection, participants were instructed to hyperventilate for 5 minutes to lower and then maintain a partial pressure of CO_2 ($PetCO_2$) at 22 ± 2 mm Hg (sea level) or 17 ± 2 mm Hg (5,050 m). Participants were then switched to the rebreathing bag at the end of expiration and were instructed to take three deep breaths to ensure rapid equalization of PCO_2 in the rebreathing circuit. The rebreathing tests were terminated when either: (1) $PetCO_2$ reached 60 mm Hg; (2) partial pressure of end-tidal O_2 ($PetO_2$) dropped below 160 mm Hg; (3) ventilatory response (VE) exceeded 100 L min^{-1}, or (4) the participant reached the end of their tolerance.

The rebreathing data were analyzed on a breath-by-breath basis using a specially designed program (Full Fit Rebreathing program, Version 3.1, University of Toronto, Toronto, Canada). In brief, the initial three-breath equilibration, sighs, swallows, and aberrant breaths were excluded from analysis. Next, the breath-by-breath $PetCO_2$ values were plotted against time and fitted with a least squares regression line to minimize interbreath variability.[26] Subsequently, VE, middle cerebral artery velocity (MCAv), cerebrovascular conductance index CVCi, Mean Arterial Pressure (MAP), and Heart Rate (HR) were plotted against the predicted $PetCO_2$ obtained by the regression analysis. The MAP was measured beat-by-beat noninvasively (Finometer, Finapres Medical Systems. The Netherlands) and confirmed via manual measures at the start of each test.

The VE plot was fitted with a model made up of the sum of two segments separated by a breakpoint.[26] The first segment was taken from resting VE. Thereafter, VE increased in conjunction with the predicted $PetCO_2$. Because hyperoxia ($PaO_2 \geq 150$ mm Hg) diminishes peripheral chemoreceptors output,[27] the observed breakpoint was taken as the ventilatory recruitment threshold of the central chemoreflex, whereas the second segment was assumed to be the ventilatory CO_2 sensitivity (or gain) attributed primarily to the central chemoreflex.

Isocapnic Hypoxia

Participants wore a nose clip and breathed through a mouthpiece connected to a Y-valve that allowed 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 for 5 minutes, before participants were switched to the rebreathing circuit. Participants filled the rebreathing bag with room air drawn in through the nose and expired into the bag. Once the bag was filled, (ensuring that this was at

the end of an expiration), the nose clip was attached and rebreathing began. This modification of the classic technique[28] avoided the possibility of a "hidden CO_2 stimulus."[29] The isocapnic hypoxia was terminated when either: (1) $PetO_2$ reached 45 mm Hg at sea level and 30 mm Hg at 5,050 m; (2) the VE exceeded 100 $Lmin^{-1}$; or (3) the participant reached the end of their tolerance.

These breath-by-breath data were plotted against $PetO_2$ and an inverse first order polynomial function was used to obtain the hypoxic ventilatory response curve[30]:

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

where, y_0 is the y asymptote, x is the $PetO_2$ in mm Hg, and c is the curvature (representing the responsiveness).

Respiratory Variables

Inspiratory flow was measured using a heated pneumotachograph (Hans-Rudolph 3813, Kansas City, MO, USA), attached to the intake valve of the mask (steady state) or to the mouthpiece, via a disposable filter (rebreathing). Pressures of end-tidal CO_2 and O_2 were sampled from the leak-free mask or from a needle inserted into the mouthpiece and measured using gas analyzers (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 analyzers were calibrated using known concentrations of O_2 and CO_2.

Cerebrovascular hemodynamics 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, Dunedin, New Zealand), interfaced with a computer, and were subsequently analyzed using commercially available software, (Chart v7, ADInstruments, Dunedin, New Zealand).

Both hyperoxic hypercapnic rebreathing tests[31] and isocapnic hypoxic[28] rebreathing tests were performed before and after drug administration, using a mask and valve system (Hans-Rudolph 7900 Series) and rebreathing circuit.

Measurements

Cerebral Blood Flow Velocity

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.[32,33] The Doppler probe was secured with a plastic headband device (Spencer Technologies, Northborough, MA, USA) to maintain optimal insonation position and angle throughout the protocol.

Cerebrovascular Reactivity

This was calculated for both hypocapnia and hypercapnia by dividing the change in MCAv by the change in $PetCO_2$ in mm Hg. Blood pressure reactivity to CO_2 was calculated by dividing the change in MAP by the change in $PetCO_2$ during the cerebrovascular reactivity testing.

Blood Gases

Arterial blood variables [pH, partial pressure of arterial O_2 (PaO_2), partial pressure of arterial CO_2 ($PaCO_2$), arterial O_2 saturation (SaO_2), bicarbonate concentration [HCO_3^-], and hematocrit (Hct)] from the radial artery were obtained after 10-minute supine rest using a 25-gauge needle into a preheparinised syringe. Following standardized calibration, all blood samples were analyzed using an arterial blood-gas analyzing system, (NPT™7 series, Radiometer, Copenhagen, Denmark).

Statistical Analysis

Data Sets

There were complete data sets for the collected variables for MCAv, arterial blood gas and polysomnography data; however, some ventilatory response test data were incomplete, because either the test failed, or the result was more than 2 SD from the mean of that group. These were uncommon events. However, there were five empty cells of 48 in the paired measurements of hypoxic ventilatory response (HVR) and hypercapnic ventilatory response (HCVR) before and after the ACZ administration and 6 empty cells from 48 in the HVR and HCVR results before and after INDO administration.

All results were analyzed using the Aabel software for the Macintosh, (Gigawiz, Louisville, KY, USA). Data sets that were normally distributed were analyzed by paired *t* test (most data). Data sets in which the data were not normally distributed (post-ACZ HVR, pre-ACZ HCVR, post-INDO HCVR) were analyzed with their data pairs by a nonparametric test (Wilcoxon signed rank test).[34]

RESULTS

Effects of Acclimatization

The mean AHI increased by approximately 50% (76.9 ± 48.9 to 115.9 ± 20.2/h) ($P = 0.01$) during the 2-week acclimatization period, as reported elsewhere.[35] Time constraints meant that a control sleep study at the same time as the interventional sleep studies was not possible, so an arithmetic mean of AHI severity from the sleep studies conducted upon arrival and after 2 weeks at high altitude was used for comparison with the intervention sleep studies. This is shown graphically in Figure 2. For all other comparisons (e.g., CBF, arterial blood gases, ventilatory responses), control measurements were made immediately before the drug administrations.

Effect of ACZ and INDO

Acetazolamide

Acute IV administration of ACZ increased awake resting MCAv by 28% (95% CI: 22-34%) ($P < 0.001$; Table 3), whereas the apnea-hypopnea index (AHI) that night was 56% (95% CI: -81 to -32%) lower than control ($P = 0.001$; Table 2). Following ACZ administration, $PaCO_2$ rose ($P < 0.001$; Table 3), which caused a slight fall in pH ($P < 0.001$; Table 3). In addition, the metabolic contribution to the pH (i.e., calculated standard base excess) became slightly less negative ($P < 0.004$; Table 2).

The HVR, as represented by "Parameter A,"[36] did not significantly change after the administration of ACZ (see Table

Table 2—The effects of altering cerebral blood flow on apnea-hypopnea index.

Subject No.	Mean Control AHI	Post ACET AHI	Post ACET % change in AHI	Post INDO AHI	Post INDO % change in AHI
1	71.4	95.8	+34.2	148.5	+108
2	150.2	131.3	-12.6	133.7	-11
3	25	3.2	-87.2	38.5	+54
4	63.2	8.8	-86.1	141.5	+123.9
5	86.9	0.4	-99.5	111.7	+28.5
6	139	62.1	-55.3	136.7	-2
7	114.6	95.9	-16.3	108.8	-5.1
8	116	84.9	-26.8	90.8	-21.7
9	45.7	7.5	-83.6	108.6	+137.6
10	149.6	72.8	-51.3	152.5	+1.9
11	67.1	3.1	-95.4	82.1	+22.4
12	41.5	0	-100	96.4	+132.3
Mean ± SD	89 ± 43.7	47.1 ± 48.1 [b]	-56.7 ± 43.2%	112.5 ± 32.9 [a]	+47 ± 61.3%

[a] $P < 0.05$. [b] $P = 0.001$ compared to mean control. AHI, apnea-hypopnea index (events/h of sleep); ACET, acetazolamide; INDO, indomethacin.

3); nor was the HCVR significantly altered from 6.6 ± 4.6 L/min/mm Hg CO_2 (P = not significant) after ACZ. Hypercapnic MCAv-CO_2 reactivity was not significantly altered following ACZ administration (3.2 versus 4.1 cm/s/mm Hg CO_2; $P > 0.10$), whereas hypocapnic MCAv-CO_2 reactivity increased from 2.7 to 4.3 cm/sec/mm Hg CO_2 ($P < 0.01$) following ACZ. There was no change in blood pressure reactivity to changes in $PetCO_2$ (1.6 versus 1.8 mm Hg MAP/mm Hg CO_2; $P = 0.87$).

Indomethacin

Ninety minutes following the oral administration of INDO, awake resting MCAv was reduced by 23% (95% CI: -16% to -30%) ($P < 0.001$; Table 3) and the average % change in AHI was +47% (95% CI: 13-82%) higher than control ($P < 0.05$; Table 2). The $PaCO_2$ did not change from 28 ± 2 mm Hg (see Table 3), yet metabolic alkalosis was still observed, with the pH rising slightly ($P < 0.001$; Table 3). At the same time, standard base excess became less negative, ($P < 0.01$; Table 3), confirming that the slight alkalosis from the INDO was metabolic in origin.

Although the HVR did not increase significantly following INDO, the HCVR was increased by 40% ($P < 0.02$; Table 3). The mean % increase was 60% (95% CI: 30-102%). The hypercapnic MCAv-CO_2 reactivity was reduced following INDO (3.4 to 2.1 cm/sec/mm Hg CO_2; $P < 0.01$), as was hypocapnic MCAv-CO_2 reactivity (2.5 to 1.1 cm/sec/mm Hg CO_2; $P < 0.01$). There was no change in blood pressure reactivity to changes in $PetCO_2$ (1.3 versus 1.2 mm Hg MAP/mm Hg CO_2; $P = 0.63$).

Table 4 shows the comparison of the effects of both drugs on AHI when compared directly to each other. There was a highly significant difference between the group AHI indices ($P < 0.01$).

Selective Correlations

Table 5 shows the correlation coefficients for the relevant respiratory variables following the administration of the two drugs and the potential influence that each had with the severity of AHI. The strongest correlations between AHI and key variables after ACZ administration were with pH, HCVR, HVR, and the blood gas variables ($PaCO_2$ and PaO_2) as one might expect. After INDO, the strongest correlation was a negative correlation with CBF, suggesting a different method of effect than ACZ on AHI. There were persisting strong correlations with pH, HVR, and the blood gas variables (PaO_2 and $PaCO_2$). The previously strong correlation with HCVR had disappeared.

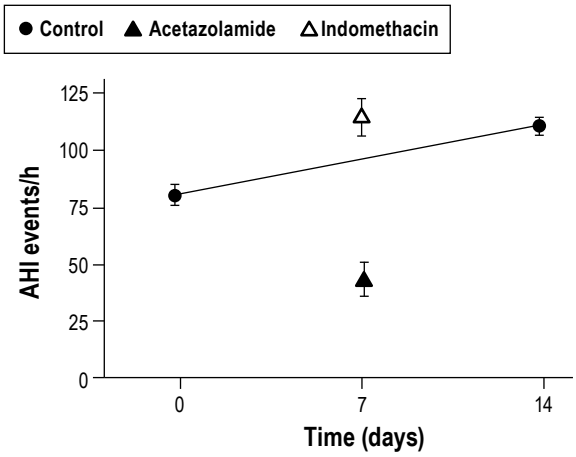

Figure 2—Central sleep apnea versus time and drug administration. Mean ± SE bars.

DISCUSSION

We report the results of what we believe to be the first attempt to artificially manipulate CBF in the field, in the midst of 2 weeks of acclimatization to an altitude of 5,050 m above sea level, in a group of otherwise healthy volunteers. Both drugs were effective in altering CBF. Intravenous ACZ, however, appears to have affected other tissues causing unintended effects, which makes the interpretation of the acetazolamide data quite complicated, and is the reason for the several caveats outlined in the discussion. The INDO administration appears to have had no unintended effects, so those results support a role for

Table 3—The effects of administration of intravenous acetazolamide and oral indomethacin.

	Pre-acetazolamide	Post-acetazolamide	Pre-indomethacin	Post-indomethacin
MCAv (cm/sec)	73.3 ± 11.9	93.6 ± 14.5 [f]	70 ± 13	53.6 ± 11.4 [f]
AHI (event/h)	89 ± 43.7	47.1 ± 48.1 [f]	89 ± 43.7	112.5 ± 32.9 [d]
PaO_2 (mm Hg)	46 ± 4	43 ± 3 [d]	47 ± 4	44 ± 3 [d]
$PaCO_2$ (mm Hg)	28 ± 4	31 ± 3 [f]	28 ± 2	28 ± 2
pH	7.45 ± 0.02	7.43 ± 0.02 [f]	7.45 ± 0.03	7.48 ± 0.04 [f]
BE	-4.3 ± 3	-2.8 ± 2.4 [e]	-4.5 ± 2.7	-2.6 ± 2.3 [e]
HCVR [a,b] (l/min/mm Hg)	6.6 ± 4.6 (n = 12)	6.1 ± 3.6 (n = 12)	6.3 ± 6.2 (n = 10)	8.2 ± 7.1 [g] (n = 12)
HVR [c] (Parameter A)	2600 ± 1700 (n = 11)	3000 ± 2300 (n = 10)	3200 ± 1900 (n = 10)	5200 ± 5400 (n = 9)

Pre drug values for AHI are arithmetic mean values of the arrival and predeparture sleep studies. All other control values recorded immediately before intervention. [a] The power of the statistical comparisons = 92% for pre acetazolamide and post- acetazolamide and 79% for pre indomethacin and post indomethacin. [b] The threshold for the central chemoreflex before and after intravenous acetazolamide was 28.3 ± 3.2 and 30.8 ± 2.8 mm Hg. (not significant; NS). The threshold before and after indomethacin was 27.6 ± 2.5 and 28.1 ± 1.8 mm Hg. (NS). [c] The power of the statistical comparisons = 86% for pre acetazolamide and post acetazolamide and 70% for pre indomethacin and post indomethacin. [d] $P < 0.05$. [e] $P < 0.01$. [f] $P \leq 0.001$. [g] $P < 0.02$. BE, base excess; HCVR, hypercapnic ventilatory response; HVR, hypoxic ventilatory response; MCAv, middle cerebral artery velocity; $PaCO_2$, partial pressure of arterial carbon dioxide; PaO_2, partial pressure of oxygen.

Table 4—Comparison of apnea-hypopnea index values between both drugs.

Subject No.	Acetazolamide 3-h AHI	Indomethacin 3-h AHI
1	95.8	148.5
2	131.3	133.7
3	3.2	38.5
4	8.8	141.5
5	0.4	111.7
6	62.1	136.7
7	95.9	108.8
8	84.9	90.8
9	7.5	108.6
10	72.8	152.5
11	3.1	82.1
12	0	96.4
Mean ± SD	47 ± 48	113 ± 33 [a]

[a] $P < 0.01$ paired *t* test. AHI, apnea-hypopnea index; SD, standard deviation.

Table 5—Correlation coefficients for relevant variables following drug administration.

	Postacetazolamide		Postindomethacin	
Inputs	r^2	P	r^2	P
AHI / CBF	0.10	< 0.01	-0.26	< 0.001
AHI / HCVR	-0.29	0.01	-0.02	< 0.001
AHI / $PaCO_2$	-0.09	NS	-0.11	< 0.001
AHI / HVR	-0.33	< 0.01	0.33	0.02
AHI / pH	0.47	0.01	0.41	< 0.001
AHI / PaO_2	0.22	NS	-0.12	< 0.001

AHI, apnea-hypopnea index (events/h sleep); CBF, cerebral blood flow; HCVR, hypercapnic ventilatory response (L/min/mm Hg); HVR, hypoxic ventilatory response (Parameter "A"); $PaCO_2$, partial pressure of arterial carbon dioxide; PaO_2, partial pressure of oxygen; r^2, Pearson correlation coefficient (the minus sign indicates a negatively sloped relationship).

CBF in the control of breathing during acclimatisation to high altitude.

Influence of Acclimatization

Within the first 3 days of arrival at 5,050 m, severe CSA was evident, with a significant increase in CBF above sea level values associated with marked hypoxemia and evidence of hyperventilation and respiratory alkalosis. Over the 2-week period of partial acclimatization, there was evidence of renal compensation with further hyperventilation and an associated increase in PaO_2. As we have previously reported, CBFv returned toward sea-level values across the 2-week period,[13] whereas both HVR and HCVR increased.[35] There was an approximate 100% increase in mean HVR and an increase in mean HCVR of approximately 35%. Therefore, recognizing that acclimatization was ongoing, and adjusting for its effects was important in the conduct of experiments and in the interpretation of the results of the current study. This was achieved by performing new arterial blood gas, ventilatory response, and CBF measurements immediately prior to drug intervention and using a mean CSA index as control for the drug effects on AHI.

CSA at high altitude occurs during light sleep, (Stages 1 and 2 non rapid eye movement sleep), in the presence of relative hypocapnia and alkalosis at sleep onset.[9] Although many studies cite the classic study by Lahiri et al.[37] to provide evidence of the relationship between HVR and periodic breathing, this relationship was largely created by the inclusion of a Sherpa group with a blunted HVR. Upon examination, there was no obvious relationship between HVR and periodic breathing within the lowlander population. This absence of a relationship between HVR was further confirmed, albeit in a subgroup (n = 5), at 6,300 and 8,050 m.[38] These findings are consistent with those of Masuyama et al., who found that two of nine mountaineers did not develop CSA at altitude despite normal values for HVR.[39] More recently, we have also reported an absence of a relationship between HVR and periodic breathing at 5,050 m.[35] In contrast, at

4,400 m in a small sample size (n = 4) it was shown that the respiratory stimulant almitrine doubled the HVR and elevated periodic breathing compared with acetazolamide or placebo.[40] A number of potential explanations exist for these discrepant and variable findings, including evidence that the hypoxic and CO_2 response are not always similar above and below eupnea[10]; differences in awake versus sleep respiratory control; variable acid-base status, and methodological differences (e.g., chemoreflex testing, natural versus simulated altitude, etc). Nevertheless, collectively these findings highlight the multifactorial complexity of periodic breathing at high altitude.

Influence of CBF on CSA Severity

Intravenous acetazolamide caused a 28% increase in CBF. This was associated with a mean fall of 56% in AHI. Our hypothesis was that these findings would be caused by a reduction in central chemoreceptor stimulation by locally produced CO_2, because of increased clearance caused by the higher CBF. A Pearson correlation analysis of the change in AHI compared to change in CBF after IV ACZ showed no significant correlation ($r^2 = 0.05$) [although there was a weak correlation between post-IV ACZ AHI and CBF ($r^2 = 0.10$)]. One would expect that the increase in CBF, along with a tendency for cerebrovascular reactivity to be elevated, would be manifest in a reduction in the ventilatory response to CO_2 (HCVR). However, HCVR (and HVR) was unchanged, when measured awake. What did change, however, were $PaCO_2$ and pH. $PaCO_2$ rose from 28 ± 4 to 31 ± 3 mm Hg and pH decreased from 7.45 ± 0.02 to 7.43 ± 0.02 because of the rise in $PaCO_2$. It should be noted that the fall in pH was not caused by a metabolic acidosis, (which occurs more slowly after oral ACZ administration), because the base excess became slightly less acidic following the IV administration. Therefore, we believe that the rise in $PaCO_2$ was caused by the effect of IV ACZ on red cell carbonic anhydrase. It has been established, at least, in more modest normobaric hypoxia, that an increase of even a few mm Hg in $PaCO_2$ would be sufficient to markedly reduce the AHI.[9,10] Thus, it seems likely that this was the mechanism of action of the IV ACZ in this instance. Other mechanisms are possible; for example, because ventilation was not measured during sleep, a reduction in ventilation causing the rise in $PaCO_2$ cannot be excluded. Whether such hypoventilation could potentially be caused by the related elevations in CBF via IV ACZ and reductions in central and peripheral chemosensitivity during sleep, remains to be established. Furthermore, IV ACZ at 10 mg/kg in normal volunteers at sea level has been shown to inhibit the peripheral chemoreceptors in the carotid body and stimulate HCVR centrally.[41] Although we found no change in HVR at high altitude after IV ACZ, this may reflect the relative insensitivity of this test and does not exclude a differential influence during sleep.

Oral INDO administration caused a 23% (95% CI: 16-30%) reduction in CBF and increased V_E-CO_2 responsiveness by 66% (95% CI: 30-102%). This was associated with an average increase in AHI of 47%. On this occasion there was no change in $PaCO_2$, although pH increased slightly from 7.45 ± 0.03 to 7.48 ± 0.04. This may have antagonized the effect of reduced CBF on central chemoreceptors, reducing the increase in AHI that otherwise might have been expected. The relationship between change in CBF and change in AHI for all subjects after oral INDO is shown in Figure 3. The obvious pattern was that of a fall in CBF being accompanied by a rise in AHI. The magnitude of change varied considerably ($r^2 = 0.26$). Those subjects with little or no change were those with very high values for AHI prior to drug administration (AHI > 100/h), which suggests that they were perhaps already close to their maximum values for AHI (e.g., subject #2).

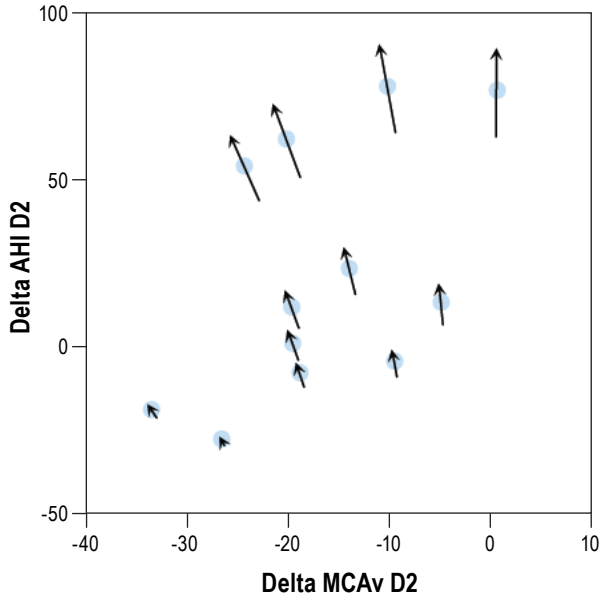

Figure 3—The relationship between cerebral blood flow velocity (CBFv) and apnea-hypopnea index (AHI) after indomethacin administration. Delta MCAv D2 = change in cerebral blood flow velocity after Indomethacin (cm/sec). Delta AHI D2 = change in apnea-hypopnea index after Indomethacin (events/hour of sleep)

As reported at sea level,[18] oral INDO was associated with significant increases in awake HCVR (up to 43%) that occurred as a result of the blunting of CBF reactivity, thus causing a delayed washout of locally produced CO_2 from around the central chemoreceptors resulting in increased stimulation; however, other direct mechanisms may have been involved. There was a non significant increase in HVR after INDO, which may have been a type II error, because the calculated power was only 70%, because of a reduced number of available data pairs (n = 9).

Limitations

The limitations of this study are that the study group comprised only 12 subjects and that acclimatization data collection was limited to 2 weeks; however, our data are broadly consistent with recent data from Bloch et al.[42] and earlier data from Salvaggio et al.[43] The inclusion of four women in the study group (with generally lower ventilatory responses) increased the scatter in the data, especially ventilatory response data. Such observations are consistent with a recent report illustrating that sex may influence the extent of periodic breathing (as indexed by AHI) at high altitude; i.e., females have a reduced AHI compared with males at 5,400 m,[44] although one of our female subjects had the highest AHI on their control night (subject #2). Because of time constraints there was no true control group in our study. Instead, approximately in the middle of the 2-week

acclimatization, in randomized order, CBF was artificially increased and decreased by drug administration. *Post hoc* analysis revealed exactly equal dispersion over time, between the two interventions within the recorded acclimatization period.

We studied only the first 3 hours of sleep because of the limited duration of effect of the INDO, which is approximately 4 hours,[21] but the first hour of effect was taken up with ventilatory response testing. We confirmed this time course by *post hoc* observation on other subjects.

Although there are a number of meaningful ways to assess the HVR at sea level using steady-state (isocapnic hypoxia) or rebreathing methods (hyperoxic versus hypoxic rebreathing), at high altitude the methodological approach becomes even more complex,[25,45,46] and consensus on the best approach has not been reached. It is known that steady state techniques produce higher values for HVR than non steady-state techniques[47] such as the modified rebreathing test we used.[28] We accept that the rebreathing test may have underestimated the true HVR; however, any systematic underestimation would apply equally to the pre drug and post drug measurements. The classic rebreathing test has also been criticized for applying a "hidden CO_2 stimulus" at the same time as the hypoxic stimulus to ventilation.[29] However, in the same journal,[48] van Klaveren and Demedts have rebutted that criticism and support the ongoing use of the rebreathing test to assess HVR. Those issues are not relevant to our measurements, however, because we used a modification of the classic test whereby the subjects began the rebreathing from a mixed expired gas mix, which would have had a PCO_2 value slightly less than alveolar PCO_2, so no "hidden CO_2 stimulus" could be present. Moreover, we aggressively allowed end-tidal PO_2 to decrease to 30 mm Hg and therefore generate a peripheral chemoreflex response for that environment. Because this was a within-subjects design, we did not need to correct HVR for vital capacity or forced expiratory volume (FEV1),[49] which has been suggested by others to improve the test.

We do accept that in a short non steady state HVR test that CBF will change during the test[50] which introduces an unmeasureable additional influence; however, because the tests were of equal length before and after drug administration, then any errors were likely common to both conditions. Thus, we believe that the use of our isocapnic hypoxic test in the context of field conditions, while not perfect, provided some index of HVR. Nevertheless, more sophisticated chemoreflex testing (e.g., end-tidal forcing methods)[50] if feasible in the field would improve our understanding in this area.

We have used MCAv measured by the transcranial Doppler technique,[32,33] which is not the same as the measurement of CBF to the vertebrobasilar system. It is, however, the current standard in this kind of research and is widely used[13]; it is also the only practical index of CBF that can be made during sleep, especially in the field at high altitude. Although the increase in CBF using IV ACZ dramatically reduced CSA, the interpretation of that outcome has been confounded by the significant increase in $PaCO_2$ (and presumably brain PCO_2) and a reduction in pH. These changes were possibly caused by paralysis of carbonic anhydrase in red blood cells, or possibly a reduction in ventilation. Thus, the increase in $PaCO_2$ may have swamped other effects, including the effect of increasing CBF.

CONCLUSION

The findings of the current study highlight an important role for CBF in CSA severity at high altitude. There were meaningful correlations between changes in AHI and pH, and between AHI and $PaCO_2$. Reducing CBF and its reactivity to CO_2 with INDO significantly increased the hypercapnic ventilatory responses and the central AHI. The AHI after indomethacin was strongly correlated with the reduction of CBF, which supports our hypothesis.

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DISCLOSURE STATEMENT

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REFERENCES

1. Krum H, Jelinek M, Stewart S, Sindone A, Atherton J, Hawkes A. Guidelines for the prevention, detection and management of people with chronic heart failure in Australia. Med J Aust 2006;185:549-56.
2. Hanly P, Millar T, Steljes D, Baert R, Frais M, Jryger M. The effect of oxygen on respiration and sleep in patients with congestive heart failure. Ann Intern Med 1989;111:777-82.
3. Bradley T, Logan A, Kimoff R, et al. Continuous positive airway pressure for central sleep apnea and heart failure. N Engl J Med 2005;353:2025-33.
4. De Backer W, Verbraecken J, Willeman M, Wittesaele W, De Cock W, Van De Heyning P. Central apnea index decreases after prolonged treatment with acetazolamide. Am J Respir Crit Care Med 1995;151:87-91.
5. Swenson E, Hughes J. Effects of acute and chronic acetazolamide on resting ventilation and ventilatory responses in men. J Appl Physiol 1993;74:230-7.

6. Berssenbrugge A, Dempsey J, Iber C, Skatrud J, Wilson P. Mechanisms of hypoxia induced periodic breathing during sleep in humans. J Physiol 1983;343:507-25.
7. Mebrate Y, Wilson K, Manisty C, et al. Dynamic CO2 therapy in periodic breathing: a modeling study to deter optimal timing and dosage regimes. J Appl Physiol 2009;107:696-706.
8. Burgess K, Johnson P, Edwards N. Central and obstructive sleep apnea during ascent to high altitude. Respirology 2004;9:222-9.
9. Dempsey J, Skatrud J. A sleep-induced apneic threshold and its consequences. Am Rev Respir Dis 1986;133:1163-70.
10. Dempsey J. Crossing the apneic threshold: causes and consequences. Exp Physiol 2005;90:13-24.
11. White D. Pathogenesis of obstructive and central sleep apnea. Am J Respir Crit Care Med 2005;172:1363-70.
12. Xie A, Skatrud J, Morgan B, et al. Influence of cerebrovascular function on the hypercapnic ventilatory response in healthy humans. J Physiol 2006;577:319-29.
13. Lucas S, Burgess K, Thomas K, et al. Alterations in cerebral blood flow and cerebrovascular reactivity during 14 days at 5050m. J Physiol 2011;589:741-53.
14. Burgess K, Burgess K, Subedi P, Ainslie P, Topor Z, Whitelaw W. Prediction of periodic breathing at altitude. Adv Exp Med Biol 2008;605:442-6.
15. Andrews G, Ainslie P, Shepherd K, et al. The effect of partial acclimatization to high altitude on loop gain and central sleep apnea severity. Respirology 2012;17:835-40.
16. Douglas N, White D, Weil J, Pickett C, Zwillich C. Hypercapnic ventilatory response in sleeping adults. Am Rev Respir Dis 1982;126:758-62.
17. Fan J-L, Burgess K, Thomas K, et al. Effects of acetazolamide on cerebrovascular function and breathing stability at 5,050m. J Physiol 2012;590(Pt 5):1213-25.
18. Fan J-L, Burgess K, Thomas K, et al. Influence of indomethacin on ventilatory and cerebrovascular responsiveness to CO2 and breathing stability: the influence of PCO2 gradients. Am J Physiol Regul Integr Comp Physiol 2010;298:R1648-58.
19. Basnyat B, Gertsch J, Holck P, et al. Acetazolamide 125 mg BD is not significantly differeent from 375 mg BD in the prevention of acute mountain sickness: the Prophylactic Acetazolamide dosage Comparison for Efficacy (PACE) trial. High Alt Med Biol 2006;7:17-27.
20. Ritschel W, Paulos C, Arancibia A, Agrawal M, Wetzelsberger K, Lucker P. Pharmacokinetics of acetazolamide in healthy volunteers after short- and long-term exposure to high altitude. J Clin Pharmacol 1998;38:533-9.
21. Xie A, Skatrud J, Khayat R, Dempsey J, Morgan B, Russell D. Cerebrovascular response to carbon dioxide in patients with congestive heart failure. Am J Respir Crit Care Med 2005;172:371-8.
22. Burgess K, Johnson P, Edwards N, Cooper J. Acute mountain sickness is associated with sleep desaturation at high altitude. Respirology 2004;9:485-92.
23. Sleep-related breathing disorder in adults: recommendations for syndrome definition and measurement techniques in clinical research. The report of an American Academy of Sleep Medicine task force. Sleep 1999;22:667-89.
24. Rechtschaffen A, Kales A. A manual of standardised terminology, techniques and scoring stages of human subjects. Brain Information Service/Brain Research Institute, University of California. Los Angeles, CA, 1990.
25. Duffin J. Measuring the respiratory chemoreflexes in humans. Respir Physiol Neurobiol 2011;177:71-9.
26. Duffin J, Mohan R, Vasiliou P, Stephenson R, Mahamed S. A model of the chemoreflex control of breathing in humans: model parameters measurement. Respir Physiol 2000;120:13-26.
27. Cunningham D, Hey E, Patrick J, Lloyd B. The effect of noradrenaline infusion on the relation between pulmonary ventilation and the alveloar PO2 and PCO2 in man. Ann N Y Acad Sci 1963;109:756-71.
28. Rebuck A, Campbell E. A clinical method for assessing the ventilatory response to hypoxia. Am Rev Respir Dis 1974;109:345-50.
29. Robbins P, Zhang S. Letter to the editor. Respir Physiol 1999;115:333.
30. Day T, Wilson R. A negative interaction between brainstem and peripheral respiratory chemoreceptors modulates peripheral chemoreflex magnitude. J Physiol 2009;587:883-96.
31. Duffin J, McEvoy G. The peripheral-chemoreceptor threshold to carbon dioxide in man. J Physiol 1988;406:15-26.
32. Aaslid R, Markwalder T, Nornes H. Noninvasive transcranial doppler, ultrasound recording of flow velocity in basal cerebral arteries. J Neurosurg 1982;57:769-74.
33. Moehring M, Spencer M. Power M-Mode Doppler (PMD) for observing cerebral blood flow and tracking emboli. Ultrasound Med Biol 2002;28:49-57.
34. Rosner B. Fundamentals of Biostatistics. Boston, MA: Duxbury Press, 1982.
35. Burgess K, Lucas S, Shepherd K, et al. Worsening of central sleep apnea at high altitude--a role for cerebrovascular function. J Appl Physiol 2013;114:1021-8.
36. Lloyd B, Jukes M, Cunningham D. The relationship between alveolar oxygen pressure and the respiratory response to carbon dioxide in man. Q J Exp Physiol Cogn Med Sci 1958;43:214-22.
37. Lahiri S, Maret K, Sherpa M. Dependence of high altitude sleep apnea on ventilatory sensitivity to hypoxia. Respir Physiol 1983;52:281-301.
38. West JB, Peters RM Jr, Aksnes G, Maret KH, Milledge JS, Schoene RB. Nocturnal periodic breathing at altitudes of 6,300 and 8,050 m. J Appl Physiol 1986;61:280-7.
39. Masuyama S, Kohchiyama S, Shinozaki T, et al. Periodic breathing at high altitude and ventilatory responses to O2 and CO2. Jpn J Physiol 1989;39:523-35.
40. Hackett P, Roach R, Harrison G, Schoene R, Mills WJ. Respiratory stimulants and sleep periodic breathing at high altitude. Almitrine versus acetazolamide. Am Rev Respir Dis 1987;135:896-8.
41. Vovk A, Duffin J, Kowalchuk J, Paterson D, Cunningham D. Changes in chemoreflex characteristics following acute carbonic anhydrase inhibition in humans at rest. Exp Physiol 2000;85:847-56.
42. Bloch K, Latshang T, Turk A, et al. Nocturnal periodic breathing during acclimatization at very high altitude at Mount Muztagh Ata (7,546m). Am J Respir Crit CareMed 2010;182:562-8.
43. Salvaggio A, Insalaco G, Marrone O, et al. Effects of high-altitude periodic breathing on sleep and arterial oxyhaemoglobin saturation. Eur Respir J 1996;12:408-13.
44. Lombardi C, Meriggi P, Agostino P, et al. High-altitude hypoxia and periodic breathing during sleep: gender-related differences. J Sleep Res 2013;22:322-30.
45. Teppema L, Dahan A. The ventilatory response to hypoxia in mammals: mechanism, measurement, and analysis. Physiol Rev 2010;90:675-754.
46. Powell FL. Measuring the respiratory chemoreflexes in humans by J. Duffin. Respir Physiol Neurobiol 2012;181:44-5.
47. Mahutte C, Rebuck A. Influence of rate of induction of hypoxia on the ventilatory response. J Physiol 1977;284:219-27.
48. Van Klaveren R, Demedts M. Response to letter to editor. Respir Physiol 1999;115:335.
49. van Klaveren R, Demedts M. Determinants of the hypercapnic and hypoxic response in normal man. Respir Physiol 1998;113:157-65.
50. Steinback C, Poulin M. Cardiovascular and cerebrovascular responses to acute isocapnic and poikilocapnic hyposic in humans. J Appl Physiol 2008;104:482-9.