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Award Number: W81XWH-11-2-0040

TITLE: AltitudeOmics: The Basic Biology of Human Acclimatization to High Altitude

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CONTRACTING ORGANIZATION: University of Colorado, Denver, Colorado 80045

REPORT DATE: Sept 2013

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE Sept 2013	**2. REPORT TYPE** Final	**3. DATES COVERED** *1 JAN 2011 - 30 JUN 2013*
4. TITLE AND SUBTITLE AltitudeOmics: The Basic Biology of Human Acclimatization to High Altitude		**5a. CONTRACT NUMBER:** **5b. GRANT NUMBER** W81XWH-11-2-0040 **5c. PROGRAM ELEMENT NUMBER**
6. AUTHOR(S) Roach, Robert, PhD		**5d. PROJECT NUMBER** **5e. TASK NUMBER** **5f. WORK UNIT NUMBER**
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Regents of the University of Colorado 13001 E. 17th Place, building 500, W1126 Aurora, CO 80045-25-5		**8. PERFORMING ORGANIZATION REPORT NUMBER**
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		**10. SPONSOR/MONITOR'S ACRONYM(S)** **11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release

13. SUPPLEMENTARY NOTES

14. ABSTRACT

For the last 12 months we have been working on data analysis and manuscript preparation. The first 6 papers from this study have been published, and another 6 are in preparation. The overview physiology paper was accepted last week at PLOS ONE, and the others have been accepted in major physiology journals. The next batch of papers will be from the OMICS portion of the study. We hired a new bioinformatics postdoc in October, and he has hit the ground running with comprehensive analyses of the OMICS dataset. The gene expression paper will be complete in another 3-4 weeks, and shortly after that the metabolomics and epigenetics data will be ready for publication. By the end of 2014 all major papers form the study will have been published. So far the project is from our perspective a complete success with identification of new physiological aspects of acclimatization, and first-ever insights into the underlying OMICS mechanisms. We are now looking for one additional year of funding to explore new analyses and integration that is possible because of the high quality of this dataset, and that could lead to even more comprehensive, "big picture" views of the process of human acclimatization to hypoxia at high altitude.

15. SUBJECT TERMS
Hypoxia, High Altitude Acclimatization

16. SECURITY CLASSIFICATION OF: U			**17. LIMITATION OF ABSTRACT UU**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**		129	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION:

The goal of this project is to advance high-altitude medical research by discovering the basic molecular mechanisms of acclimatization and de-acclimatization that protect soldiers from high-altitude illness.

BODY:

All major milestones have been accomplished. Now we are working on papers integrating the findings from the extensive physiological studies and the OMICS studies. Since no one has done that work before, we are inventing the methods and approaches as we go along. A major breakthrough ahs been the application of an advanced clustering algorithm called WGCNA to our datasets. This will allow us to condense the enormous datasets generated by the gene expression and epigenetics chip studies into a manageable system that can easily be tested for relationships to physiological tests.

Accomplishments to date:

- IRB compliance and continuing review have been completed
- Analyses are completed for all subjects at all time points for epigenetics, gene expression, microRNA and metabolomics.
- All cytokine arrays are done, with follow-up and validation ELISAs completed. Writing of those manuscripts is underway.
- Six papers have been accepted for publication, three in Journal of Applied Physiology, one in Experimental Physiology, one in Acta Scandinavica and the overview paper at PLOS ONE
- A seventh paper is under review at NeuroReports.
- Nitric oxide analyses are done, adenosine and hydrogen sulfide analyses are done. Work has begun on a paper on NO and H2S with Drs. Roach, Kevil and Gladwin.
- Analysis of ADP, ATP and purigenic receptors is complete, writing that manuscript is underway. Another paper is in the works as well with Drs. Eltzschig, Blackburn, Xia, and Davis on adenosine in AltitudeOmics.
- The Lovering laboratory, home of our collaborators on AltitudeOmics, have two papers in preparation on AMS and intrapulmonary shunts, and one on gas exchange during AltitudeOmics.

KEY RESEARCH ACCOMPLISHMENTS:

1. Completed the first ever measurements of acute mountain sickness, cognitive function and exercise capacity after 7 and 21 days of de-acclimatization. The results suggest near complete retention of acclimatization after 7 days de-acclimatization, and about 70% retention after 21 days. This key finding will be used in the OMICS analyses to help identify factors that occur with acclimatization, and are still present after de-acclimatization.
2. Six research papers have been completed and published on the physiology of human acclimatization to high altitude, and another is under review. Seven additional primary papers will be completed this year. Please see Appendices section for a table showing the "Status of Research Papers" and for a PDF of the published papers.

REPORTABLE OUTCOMES:

1. Completed all regulatory steps to gain approval for this multi-site, multi-nation study.
2. Safely completed data collection on 23 young healthy student volunteers, and safely transported and cared for them and 40 scientists to/from Bolivia.
3. We are 100% in analysis and manuscript writing mode regarding all aspects of the study.

CONCLUSION:

Humans retain acclimatization after 7 and 21 days of de-acclimatization. This was a key hypothesis of the study. Yet to be determined is what are the OMICS responses that can be linked to the process of gaining acclimatization, and its retention on descent to low altitude?

Status of manuscripts from the AltitudeOmics study, 2/15/2014

#	Title	First/Last Authors	Status	PMID	Link to PDF
1	Amann M, Goodall S, Twomey R, Subudhi AW, Lovering AT, Roach AltitudeOmics: on the consequences of high-altitude acclimatizati the development of fatigue during locomotor exercise in humans. Journal of applied physiology. 2013;115:634-42.	Amman/Roach	Published, JAPPL	23813531	
2	Goodall S, Twomey R, Amann M, Ross EZ, Lovering AT, Romer LM, Subudhi AW, Roach RC. AltitudeOmics: Exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatisation to high altitude. Acta Physiologica. 2014.	Goodall/Roach	Accepted, Acta Scandinavica	24450855	
3	Subudhi AW, Fan JL, Evero O, Bourdillon N, Kayser B, Julian CG, Lovering AT, Roach RC. AltitudeOmics: Effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery. Experimental Physiology. 2013.	Subudhi/Roach	Accepted, Exp Physiol	24243839	
4	Fan JL, Subudhi AW, Evero O, Bourdillon N, Kayser B, Lovering AT, Roach RC. AltitudeOmics: Enhanced cerebrovascular reactivity and ventilatory response to CO2 with high altitude acclimatisation and re-exposure. Journal of Applied Physiology. 2013.	Fan/Roach	Accepted, JAPPL	24356520	
5	Subudhi AW, Fan JL, Evero O, Bourdillon N, Kayser B, Julian CG, Lovering AT, Panerai RB, Roach RC. AltitudeOmics: Cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness. Journal of Applied Physiology. 2013.	Subudhi/Roach	Accepted, JAPPL	24371013	
6	Subudhi AW, Bucher J, Bourdillon N, Davis C, Elliott J, Eutermoster M, Evero O, Fan JL, Jameson-Van Houten S, Julian CG, Kark J, Kark S, Kayser B, Kern JP, Kim SE, Lathan C, Laurie SS, Lovering AT, Paterson R, Polaner D, Ryan BJ, Spira J, Tsao JW, Wachsmuth NB, Roach RC. AltitudeOmics: The Integrative Physiology of the Onset and Retention of Acclimatization to Hypoxia in Humans. PLOS One (In Press) 2014.	Subudhi/Roach	Accepted, PLOSOne		

#	Title	First/Last Authors	Status	PMID	Link to PDF
7	AltitudeOmics: hemoglobin mass increases within 7 days of acclimatization to 5260m and is lost within 7 days of descent to 1525m	Ryan/Roach	Under revision		
8	AltitudeOmics: Detecting high altitude cognitive impairment with DANA, an Android-based neurocognitive assessment tool	Roach/Roach	Submitted, Neuroreport		
9	AltitudeOmics: Gene expression and epigenetics during ascent, acclimatization, and re-exposure to 5,260m	Julian/Roach	In prep		
10	AltitudeOmics: Effect of ascent, acclimatization, and re-exposure to 5,260m on pulmonary shunt	Elliot/Roach	In prep		
11	AltitudeOmics: Effect of ascent, acclimatization, and re-exposure to 5,260m on muscle mitochondrial respiration	Chicco/Roach	In prep		
12	AltitudeOmics: Metabolomics during ascent, acclimatization, and re-exposure to 5,260m	Monte/Roach	In prep		
13	AltitudeOmics: Effect of ascent, acclimatization, and re-exposure to 5,260m on AMS and pulmonary shunt	Kern/Roach	In prep		
14	AltitudeOmics: Gene Expression and Acute Mountain Sickness	?/Roach	In prep		
15	AltitudeOmics: MicroRNA expression during ascent, acclimatization, and re-exposure to 5,260m	Kern/Roach	In prep		

J Appl Physiol 115: 634–642, 2013.
First published June 27, 2013; doi:10.1152/japplphysiol.00606.2013.

AltitudeOmics: on the consequences of high-altitude acclimatization for the development of fatigue during locomotor exercise in humans

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[1]*Department of Medicine, University of Utah, Salt Lake City, Utah;* [2]*Faculty of Health and Life Sciences, Northumbria University, Newcastle, United Kingdom;* [3]*School of Sport and Service Management, University of Brighton, Eastbourne, United Kingdom;* [4]*Altitude Research Center, Department of Emergency Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado;* [5]*Department of Biology, University of Colorado, Colorado Springs, Colorado; and* [6]*Department of Human Physiology, University of Oregon, Eugene, Oregon*

Submitted 20 May 2013; accepted in final form 24 June 2013

Amann M, Goodall S, Twomey R, Subudhi AW, Lovering AT, Roach RC. AltitudeOmics: on the consequences of high-altitude acclimatization for the development of fatigue during locomotor exercise in humans. *J Appl Physiol* 115: 634–642, 2013. First published June 27, 2013; doi:10.1152/japplphysiol.00606.2013.—The development of muscle fatigue is oxygen (O_2)-delivery sensitive [arterial O_2 content (C_aO_2) × limb blood flow (Q_L)]. Locomotor exercise in acute hypoxia (AH) is, compared with sea level (SL), associated with reduced C_aO_2 and exaggerated inspiratory muscle work (W_{insp}), which impairs Q_L, both of which exacerbate fatigue individually by compromising O_2 delivery. Since chronic hypoxia (CH) normalizes C_aO_2 but exacerbates W_{insp}, we investigated the consequences of a 14-day exposure to high altitude on exercise-induced locomotor muscle fatigue. Eight subjects performed the identical constant-load cycling exercise (138 ± 14 W; 11 ± 1 min) at SL (partial pressure of inspired O_2, 147.1 ± 0.5 Torr), in AH (73.8 ± 0.2 Torr), and in CH (75.7 ± 0.1 Torr). Peripheral fatigue was expressed as pre- to postexercise percent reduction in electrically evoked potentiated quadriceps twitch force ($\Delta Q_{tw,pot}$). Central fatigue was expressed as the exercise-induced percent decrease in voluntary muscle activation (ΔVA). Resting C_aO_2 at SL and CH was similar, but C_aO_2 in AH was lower compared with SL and CH (17.3 ± 0.5, 19.3 ± 0.7, 20.3 ± 1.3 ml O_2/dl, respectively). W_{insp} during exercise increased with acclimatization (SL: 387 ± 36, AH: 503 ± 53, CH: 608 ± 67 $cmH_2O \cdot s^{-1} \cdot min^{-1}$; $P < 0.01$). Exercise at SL did not induce central or peripheral fatigue. $\Delta Q_{tw,pot}$ was significant but similar in AH and CH (21 ± 2% and 19 ± 3%; $P = 0.24$). ΔVA was significant in both hypoxic conditions but smaller in CH vs. AH (4 ± 1% vs. 8 ± 2%; $P < 0.05$). In conclusion, acclimatization to severe altitude does not attenuate the substantial impact of hypoxia on the development of peripheral fatigue. In contrast, acclimatization attenuates, but does not eliminate, the exacerbation of central fatigue associated with exercise in severe AH.

altitude; respiratory muscle work; arterial O_2 content; cerebral blood flow

THE DEVELOPMENT OF LOCOMOTOR muscle fatigue during whole-body endurance exercise is highly sensitive to the delivery of oxygen [O_2; arterial O_2 content (C_aO_2) × leg blood flow (Q_L)]. Specifically, blunted O_2 delivery exaggerates, and augmented O_2 delivery attenuates the rate of development of locomotor muscle fatigue during exercise (1).

Acute exposure to hypoxia (AH) has a substantial impact on the two determinants of leg muscle O_2 delivery during strenuous locomotor exercise. First, despite a marked hyperventilatory response, arterial partial pressure of O_2 [PO_2 (P_aO_2)] and arterial hemoglobin saturation (S_aO_2) fall below sea level (SL) values and cause a significant reduction in C_aO_2. In addition, inspiratory muscle work (W_{insp}) is increased substantially at any given workload in hypoxia (2, 58), and these high levels of W_{insp} compromise, in a dose-dependent manner, Q_L during exercise (34). Each of these two determinants of leg muscle O_2 delivery, namely C_aO_2 and Q_L, accounts for, substantially and independently, the accelerated development of locomotor muscle fatigue in hypoxia (2).

During prolonged exposure to altitude, a progressive, time-dependent hyperventilation, which increases alveolar PO_2, occurs over the initial hours and days and advances more gradually over the ensuing 1–2 wk of acclimatization (56). This ventilatory acclimatization adds to an accompanying reduction in the alveolar-arterial O_2 gradient, which combined, substantially improves arterial oxygenation during exercise by increasing P_aO_2 and S_aO_2 (9, 13). Furthermore, chronic exposure to hypoxia (CH) is accompanied by erythropoiesis, and the combination of an increased hemoglobin concentration ([Hb]) plus improved oxygenation may serve to restore resting SL C_aO_2 (8, 13). In contrast to this beneficial effect on O_2 delivery, Q_L, during intense leg exercise at a given submaximal absolute workload, has been suggested to decline from SL to CH (8, 49, 64). The net effect of these acclimatization-induced, opposing consequences on leg O_2 delivery depends on the degree to which the increase in C_aO_2 can counterbalance potential reductions in Q_L. It has been documented previously that at a given absolute workload, locomotor muscle O_2 delivery is reduced from SL to AH with no further changes following acclimatization (Pikes Peak, 4,300 m) (8, 64). Therefore, given the critical role of muscle O_2 delivery in the development of fatigue, it could be argued that peripheral fatigue during constant-load endurance exercise is exacerbated in AH (vs. SL) and does not improve further during prolonged acclimatization. On the other hand, studies conducted at the same location as the present experiments [Mt. Chacaltaya (Bolivia), 5,260 m] document a reduction in locomotor muscle O_2 delivery from SL to AH and a full recovery following prolonged exposure, with the net effect of similar values in SL and CH (13). Based on these findings, it could be argued that the development of peripheral fatigue during constant-load endurance exercise is fastened in AH but recovers to SL values in CH.

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In this study, we sought to quantify exercise-induced locomotor muscle fatigue induced by the identical constant-load cycling trial performed at SL, in AH, and in CH (following 14 days at 5,260 m) to clarify the effects of acclimatization. We hypothesized that fatigue is, compared with SL, exacerbated significantly in AH and that altitude acclimatization would alleviate this impact.

METHODS

This study was conducted as part of the AltitudeOmics project, examining the integrative physiology of human responses to hypoxia. All procedures conformed to the Declaration of Helsinki and were approved by the Universities of Colorado, Oregon, and Utah Institutional Review Boards and the U.S. Department of Defense Human Research Protection Program Office. All subjects were born and raised below 1,500 m and had not traveled to elevations >1,000 m for 3 mo before the experiments. Eight subjects (age 21 ± 1 y, body weight 69 ± 11 kg, height 176 ± 10 cm) were studied at SL and following 14 days of altitude acclimatization at 5,260 m on Mt. Chacaltaya. At high altitude, subjects did not follow a systematic exercise-training program but were given the opportunity to participate, on a voluntary basis, in light hikes around the campsite (no significant change in altitude).

Experimental Protocol

All participants were familiarized thoroughly with various experimental procedures involved in this investigation. The SL experiments of the present study were conducted ~130 m above SL [Eugene, OR; barometric pressure (BP) 750.0 ± 2.2 Torr]. The experiments in AH were conducted at the same altitude, while breathing a gas mixture containing 10.5% O_2 balance nitrogen, and experiments in CH were conducted on the 14th day of acclimatization at 5,260 m (BP 408.9 ± 0.7 Torr). Two participants were tested every morning. To assure that all subjects were tested exactly on *day 14* after arrival on the mountain, the groups' transport to the mountain was staged, i.e., two new participants arrived every day. SL peak power output (W_{peak}) was obtained from a maximal incremental exercise test (70, 100, 130, and 160 W for 3 min, each followed by 15 W/min increases thereafter) on a computer-controlled bicycle ergometer (Velotron, Dynafit; RacerMate, Seattle, WA). The experimental trial consisted of the identical constant-load cycling exercise (same absolute workload and duration) in each condition. Preliminary experiments (using different subjects), conducted to identify a workload that causes voluntary exhaustion between 8 and 12 min when acutely exposed to 5,260 m, revealed that a constant workload equal to 50% of SL W_{peak} was required to reach this goal. Based on this, the workload during the experimental trials was set to equal 50% (138 ± 14 W) of the subjects' SL W_{peak} (275 ± 14 W). Since an individual's endurance/aerobic capacity is lowest in AH (vs. SL and CH) (13), the first trial was performed to voluntary exhaustion in AH, and the achieved time (10.6 ± 0.7 min) was then used for all subsequent trials. A 5-min warm-up at 10% W_{peak} (27 ± 8 W) preceded each trial. Throughout exercise, subjects were instructed to maintain their preferred pedal frequency, as determined during the practice sessions (88 ± 3 rpm). Neuromuscular function was assessed before and within 2.5 min after exercise. During these procedures, subjects breathed ambient air at SL and in CH and a gas mixture (10.5% O_2) in AH.

Exercise Responses

Pulmonary ventilation (V_E) and gas exchange were measured at rest and throughout exercise using an open circuit system (Ultima PFX; Medical Graphics, St. Paul, MN, and O2cap; Oxigraf, Mountain View, CA). Arterial O_2 saturation (S_pO_2) was estimated continuously at rest and during exercise using a pulse oximeter (Nellcor N-200; Pleasanton, CA) with adhesive forehead sensors. A correction factor based on arterial blood gases was used to adjust for the nonlinearity associated with the obtained pulse oximeter values (error between 60% and 80% saturation: 6%; error between >90% saturation: 3%). Heart rate was measured from the R–R interval of an ECG, using a three-lead arrangement. Ratings of perceived exertion were obtained using Borg's modified CR10 scale (10). [Hb] was measured (Radiometer OSM-3) in resting arterial blood samples collected at SL and on the 16th day at 5,260 m. C_aO_2 was estimated as 1.39 [Hb] × (S_pO_2/100). During all constant workload trials, esophageal pressure (P_{es}) was measured via a nasopharyngeal balloon (Cooper Surgical, Trumbull, CT), using standard procedures (7). To estimate W_{insp}, P_{es} was integrated over the period of inspiratory flow, and the results were multiplied by respiratory frequency (f_R) and labeled the inspiratory muscle pressure-time product. Vastus lateralis oxygenation was assessed using a multichannel near-infrared spectroscopy (NIRS) instrument (Oxymon Mk III; Artinis, Zetten, The Netherlands). As described previously (5), a NIR emitter and detector pair was affixed over the belly of the left vastus lateralis muscle (~15 cm proximal and 5 cm lateral to the midline of the superior border of the patella), using a spacer with an optode distance of 5.0 cm. Probes were secured to the skin using double-sided tape and shielded from light using elastic bandages. The Beer-Lambert Law was used to calculate micrometer changes in tissue oxygenation [oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb)] across time. using received optical densities from two continuous wavelengths of NIR light (780 and 850 nm) and a fixed differential path-length factor of 4.95 (26). Total hemoglobin (THb) was calculated as the sum of [O_2Hb] and [HHb] changes to give an index of change in regional blood volume (59). Data were recorded continuously at 10 Hz and expressed relative to the resting baseline recorded in each experimental condition. Mean cerebral blood flow (CBF) was estimated from blood velocity (CBFv) in the left middle cerebral artery (MCA; 50 ± 4 mm deep), determined using a 2-MHz transcranial Doppler (Spencer Technologies, Seattle, WA). An index of cerebral O_2 delivery was calculated as the product of CBFv and C_aO_2. Changes in CBFv were assumed to reflect changes in CBF, based on evidence that the MCA changes minimally in response to hypoxia and hypocapnia (47, 54). The validity of this assumption at altitude has been challenged recently (62). Evidence of MCA dilation was demonstrated in subjects at altitudes above 6,400 m, but no changes in MCA diameter were observed at altitudes comparable with the present study (<5,300 m) (63). We acknowledge that these measurements must be interpreted with caution until definitive studies of MCA diameter at altitude are conducted.

Expiratory Flow Limitations and Lung Volume Responses

Expiratory flow limitations. Subjects performed three maximal volitional flow-volume (FV) maneuvers before and after exercise (after assessment of neuromuscular function). Exercise tidal FV loops (FVLs) were plotted within the best of the six maximal loops (MFVLs), based on measured inspiratory capacity (IC) maneuvers (rest, 3 min of exercise, and immediately before the termination of exercise). Acceptable IC maneuvers during exercise required that peak inspiratory P_{es} match that obtained at rest. The amount of expiratory flow limitation was defined as the percentage of the tidal volume (V_T) that met the boundary of the expiratory portion of the MFVL (38).

Lung volumes. Functional residual capacity (FRC) was measured in a body plethysmograph (Platinum Elite Series; Medical Graphics), and total lung capacity (TLC) was calculated as the sum of FRC and IC. End-expiratory lung volume (EELV) was determined by subtracting the maximal IC, as measured during exercise from TLC, as measured at rest. End-inspiratory lung volume (EILV) was calculated as the sum of EELV and V_T. Inspiratory reserve volume, during exercise, was calculated by subtracting EILV from TLC, and expiratory reserve volume, during exercise, was determined by subtracting the residual volume from EELV.

Force and Compound Muscle Action Potentials

Knee-extensor force during voluntary and evoked contractions was measured using a calibrated load cell (Tedea, Basingstoke, UK). The load cell was fixed to a custom-built chair and connected to a noncompliant cuff, attached around the participant's right leg, just superior to the ankle malleoli. Participants sat upright in the chair with the hips and knees at 90° of flexion. Compound muscle action potentials (M-waves) were recorded from surface electrodes placed 2 cm apart over the vastus lateralis muscle belly. A reference electrode was placed over the patella. Evoked signals were amplified [gain: 1,000; force: custom-built bridge amplifier; electromyographic (EMG): PowerLab 26T; ADInstruments (Oxfordshire, UK)], band-pass filtered (EMG only: 20–2,000 Hz), digitized (4 kHz; PowerLab 26T, ADInstruments), acquired, and later analyzed (LabChart v7.0; ADInstruments) for peak-to-peak amplitude.

Neuromuscular Function

Force and EMG variables were assessed before and immediately (<2.5 min) after each trial. Before each trial, maximum voluntary contraction (MVC) force was determined from three control contractions. Femoral nerve stimulation was delivered during each 5-s MVC, and an additional stimulus was delivered after the MVC to determine the potentiated quadriceps twitch force ($Q_{tw,pot}$) and voluntary muscle activation (VA) (42). Briefly, the force produced during the superimposed twitch (SIT), delivered within 0.5 s of attaining peak force during the MVC, was to be compared with the force produced by the single twitch, delivered during relaxation, ~2 s after the MVC: VA (%) = $[1 - (SIT/Q_{tw,pot})] \times 100$. The contraction sets were repeated three times, with 30 s between each set. Visual feedback of the target force was provided via a computer monitor.

Femoral nerve stimulation. Single electrical stimuli (200 μs pulse width) were delivered to the right femoral nerve via surface electrodes (32 mm diameter; CF3200; Nidd Valley Medical, North Yorkshire, UK) and a constant-current stimulator (DS7AH; Digitimer, Welwyn Garden City, Hertfordshire, UK). The cathode was positioned over the nerve, high in the femoral triangle; the anode was placed midway between the greater trochanter and the iliac crest (32). The site of stimulation that produced the largest resting twitch amplitude and M-wave was located. Single stimuli were delivered, beginning at 100 mA and increasing by 20 mA, until plateaus occurred in twitch amplitude and M-wave. Supramaximal stimulation was ensured by increasing the final intensity by 30% (mean current, 250 ± 55 mA). Muscle contractility was assessed for each potentiated twitch as twitch amplitude ($Q_{tw,pot}$: peak force − onset force), maximum rate of force development (MRFD), contraction time, maximum relaxation rate (MRR), and one-half relaxation time ($RT_{0.5}$). Sarcolemmal membrane excitability was inferred from the peak-to-peak amplitude of the electrically evoked M-wave (27).

Reliability Measures

On a separate day, measures of neuromuscular function were repeated twice in all subjects at SL. The two assessment procedures were separated by a 2-min walk around the laboratory, followed by a 5-min rest period. Coefficient of variation (CV) and Pearson product-moment correlation coefficients (r) were calculated to evaluate test-retest error (precision) and test-retest reliability of the neuromuscular function-assessment procedure. All correlations were significant and indicated; in combination with the CVs, acceptable degrees of reproducibility include: MVC, CV = 3.1%, $r = 0.97$; $Q_{tw,pot}$, CV = 4.1%, $r = 0.98$; M-wave peak, CV = 4.8%, $r = 0.98$; VA, CV = 3.3%, $r = 0.77$.

Statistical Analysis

A one-way repeated-measures ANOVA was performed to evaluate differences among trials. A least-significance difference test identified the means that were significantly different with $P < 0.05$. Results are expressed as mean ± SE.

RESULTS

C_aO_2 and Cerebral O_2 Delivery

C_aO_2 at rest was significantly lower in AH compared with SL and CH (17.3 ± 0.5, 19.3 ± 0.7, 20.3 ± 1.3 ml O_2/dl, respectively). Acclimatization to altitude significantly increased [Hb] and S_pO_2, resulting in similar C_aO_2 at SL and in CH ($P = 0.16$). Resting CBFv was similar among SL, AH, and CH (50.5 ± 3.7, 52.7 ± 2.3, and 55.7 ± 3.0 cm/s, respectively; $P = 0.45$). In all three conditions, CBFv increased significantly from rest to the final minute of exercise (22 ± 3%, 39 ± 6%, and 28 ± 5% for SL, AH, and CH, respectively; Table 1). The percent increase was significantly greater in AH compared with that observed at SL and in CH. The cerebral O_2 delivery index during the last minute of exercise was 18 ± 5% lower in AH vs. SL (Table 1) and 17 ± 8% greater in CH vs. SL (Table 1).

Ventilatory Effects

Ventilatory response. AH increased W_{insp} work by 34 ± 8% above that at SL ($P < 0.01$) and dropped S_pO_2 by 36 ± 3% during the final minute of exercise. Following 14 days of acclimatization, W_{insp} was increased further by 23 ± 8% from AH, and S_pO_2, during the final minute of exercise, was 36 ± 5% higher in CH vs. AH. Breathing frequency and V_E rose

Table 1. *Mean responses to the final minute of exercise (138 ± 14 W, 10.6 ± 0.7 min)*

	Sea Level	Acute Hypoxia	Chronic Hypoxia
HR, beats/min	152 ± 5	174 ± 4*	166 ± 4*†
V_E, l min^{-1}	64 ± 4	113 ± 8*	133 ± 10*†
f_R, breaths min^{-1}	32 ± 2	50 ± 3*	54 ± 3*
V_T, liter	2.0 ± 0.1	2.2 ± 0.2	2.6 ± 0.2*†
$\dot{V}O_2$, l min^{-1}	2.58 ± 0.19	2.44 ± 0.19*	2.39 ± 0.16*†
$\dot{V}CO_2$, l min^{-1}	2.51 ± 0.22	2.81 ± 0.21*	2.40 ± 0.15*†
$V_E/\dot{V}O_2$	25 ± 1	50 ± 4*	56 ± 3*†
$V_E/\dot{V}CO_2$	26 ± 1	41 ± 2*	58 ± 3*†
S_pO_2, %	94.1 ± 1.0	62.2 ± 1.8*	75.6 ± 1.2*†
CBFv, cm/s	59.1 ± 4.8	74.2 ± 3.8*	73.2 ± 3.4*
Cerebral O_2 delivery, a.u.	1,105 ± 62	895 ± 40*	1,289 ± 42*†
T_i/T_{tot}	0.35 ± 0.01	0.39 ± 0.01*	0.39 ± 0.01*
T_e, s	1.30 ± 0.08	0.74 ± 0.05*	0.70 ± 0.04*
W_{insp}, cmH$_2$O · s^{-1} · min^{-1}	387 ± 36	503 ± 53*	608 ± 67*†
IC, liter	3.29 ± 0.22	3.13 ± 0.23	3.60 ± 0.23*
V_T/IC	0.60 ± 0.03	0.68 ± 0.02*	0.72 ± 0.02*†
IRV, liter	1.30 ± 0.14	0.99 ± 0.13*	0.96 ± 0.05*
ERV, liter	1.98 ± 0.25	2.14 ± 0.29	1.67 ± 0.25*†
EILV, %TLC	80.5 ± 1.6	85.4 ± 1.7*	85.2 ± 0.9*
EELV, %TLC	51.5 ± 1.8	53.8 ± 2.5	46.9 ± 2.1*†
Expiratory flow limitation, n out of 8 subjects	0/8	2/8	4/8
RPE	12.3 ± 1.0	19.8 ± 0.1*	17.9 ± 0.6*†
Dyspnea	11.5 ± 0.7	19.5 ± 0.2*	19.3 ± 0.2*

HR, heart rate; V_E, minute ventilation; f_R, breathing frequency; V_T, tidal volume; $\dot{V}O_2$, maximum oxygen (O_2) uptake; $\dot{V}CO_2$, carbon dioxide production; S_pO_2, arterial O_2 saturation; CBFv, cerebral blood flow velocity; T_i, duration of inspiration; T_{tot}, duration of entire breath; T_e, duration of expiration; W_{insp}, inspiratory muscle work; IC, inspiratory capacity; IRV, inspiratory reserve volume; ERV, expiratory reserve volume; EILV, end-inspiratory lung volume; TLC, total lung capacity; EELV, end-expiratory lung volume; RPE, rating of perceived exertion. *$P < 0.05$ vs. sea level; †$P < 0.05$ vs. acute hypoxia, $n = 8$.

substantially over the time of exercise in AH and CH, and V_E was, during the final minute, 79 ± 13% and 110 ± 12%, respectively, higher compared with SL ($P < 0.01$). Pulmonary V_E during the final minute of exercise was 19 ± 4% higher in CH vs. AH ($P < 0.01$). Compared with SL, O_2 uptake, during the final minute of exercise, was 5 ± 2% and 7 ± 2% lower in AH and CH, respectively (both $P < 0.05$; Fig. 1).

Expiratory flow limitation. At SL, exercise flow rates during tidal breathing were well within the MFVL in all eight subjects. At end-exercise in AH, 6–51% of the V_T in two of the eight subjects reached flow limitation, as lung volume approached end-expiration. As V_E increased further in CH, expiratory flow rate became more limited, and 10–64% of the V_T in four of the eight subjects met the limit imposed by the MFVL.

Membrane Excitability and Contractile Function

M-waves. As a measure of membrane excitability we examined pre- vs. postexercise vastus lateralis M-wave amplitudes in conjunction with the quadriceps muscle mechanical properties. Pre-exercise M-wave amplitudes were similar in all three conditions (10.2 ± 1.0 mV, 9.4 ± 0.7 mV, and 12.9 ± 1.8 mV for SL, AH, and CH, respectively; $P = 0.15$). Postexercise M-wave amplitudes were unchanged from pre-exercise baseline values at SL and in AH (10.2 ± 1.0 mV and 9.6 ± 0.9 mV, respectively; $P > 0.3$). However, following exercise in CH, M-wave amplitudes (7.8 ± 2.1 mV) were reduced significantly from pre-exercise baseline levels (range: 1–18%; $P < 0.01$).

Quadriceps twitch force. Pre-exercise $Q_{tw,pot}$ was similar in all three conditions (106 ± 4 N, 109 ± 4 N, and 110 ± 5 N for SL, AH, and CH, respectively; $P = 0.18$). Exercise in both hypoxic conditions caused a substantial ($P < 0.01$) but similar ($P = 0.14$) reduction in $Q_{tw,pot}$ in all eight subjects. In contrast, exercise at SL did not induce measurable locomotor muscle fatigue; the postexercise $Q_{tw,pot}$ was similar to pre-exercise baseline.

MVC force. Pre-exercise MVC was similar in all three conditions (391 ± 30 N, 394 ± 25 N, and 372 ± 30 N for SL, AH, and CH, respectively; $P = 0.21$). At SL, postexercise MVC was similar to pre-exercise baseline ($P = 0.42$). In contrast, exercise in AH and CH caused a substantial reduction in MVC in all eight subjects. However, the exercise-induced reduction in MVC was 30 ± 9% less in CH vs. AH ($P < 0.05$).

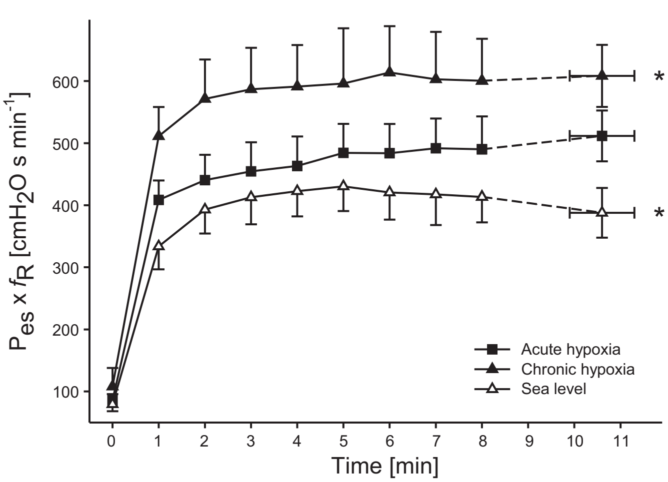

Fig. 1. Inspiratory muscle pressure-time product [esophageal pressure (P_{es}) × respiratory frequency (f_R)] during the identical constant-load cycling exercise performed in all 3 conditions. *$P < 0.05$ vs. acute hypoxia (AH), $n = 8$.

Muscle activation. Pre-exercise baseline values were similar in all three conditions (94 ± 1%, 94 ± 1%, and 93 ± 1% for SL, AH, and CH, respectively; $P = 0.19$). Following the exercise at SL, muscle activation was unchanged from pre-exercise baseline ($P = 0.88$). In both AH and CH, postexercise muscle activation was significantly lower compared with pre-exercise baseline values. However, the pre- to postexercise decrease in muscle activation was 52 ± 12% less in CH vs. AH ($P < 0.01$).

Within-twitch measurements. MRFD, MRR, and $RT_{0.5}$ complement the findings reported for $Q_{tw,pot}$. The pre- to postexercise changes in within-twitch measurements of MRFD, MRR, and $RT_{0.5}$ were similar in CH vs. AH.

Vastus Lateralis Tissue Oxygenation

O_2Hb was unchanged from baseline to warm-up at SL ($P = 0.40$) but decreased in AH ($P < 0.05$) and CH ($P = 0.05$). Compared with baseline, O_2Hb was unchanged during the final minute of exercise at SL ($P = 0.73$) but was significantly lower in AH and CH (both $P < 0.01$). This decrease was significantly greater in AH vs. CH. HHb was unchanged from baseline to warm-up at SL ($P = 0.80$) but decreased significantly in AH and CH. Compared with baseline, HHb was unchanged during the final minute of exercise at SL ($P = 0.24$) but similarly increased in AH and CH (both $P < 0.01$). THb was unchanged from baseline to warm-up in all three conditions. In contrast, compared with baseline, THb was increased significantly and similarly ($P = 0.37$) during the final minute of exercise in all three conditions.

DISCUSSION

The purpose of this investigation was to evaluate the effect of altitude acclimatization on the development of fatigue during whole-body endurance exercise. Subjects repeated the identical constant-load cycling exercise at SL, in AH, and in CH. No measurable degree of fatigue was found following the exercise at SL. However, the identical exercise in AH, characterized by a reduced C_aO_2 and increased W_{insp}, resulted in a substantial degree of both peripheral and central fatigue. Two weeks of exposure to 5,260 m restored C_aO_2 to SL values but increased W_{insp} further over that observed in AH. The critical finding was that the rate of development of peripheral locomotor muscle fatigue failed to recover from AH to CH and was similar in both conditions. In contrast, the development of central fatigue was attenuated significantly in CH (vs. AH) but still greater compared with SL. Taken together, our findings suggest that acclimatization to high altitude attenuates the impact of AH on the development of central fatigue but fails to improve the exacerbated development of peripheral fatigue present during exercise in AH.

Peripheral Fatigue

Acute hypoxia. The cycling bout in AH was, compared with SL, characterized by a substantially exaggerated rate of peripheral fatigue (Table 2 and Fig. 2). These observations confirm numerous earlier findings using whole-body (4, 31, 57) and single-muscle exercise (28, 39).

Table 2. *Effects of constant-load cycling exercise on quadriceps muscle function*

	Percent Change from Pre- to Immediately Postexercise		
	Sea Level	Acute Hypoxia	Chronic Hypoxia
$Q_{tw,pot}$	−3.1 ± 1.8*	−20.9 ± 2.4	−18.8 ± 3.4
MRFD	−4.1 ± 2.5*	−21.2 ± 4.2	−17.9 ± 3.5
MRR	2.7 ± 2.8*	−13.2 ± 3.1	−9.0 ± 2.2
$RT_{0.5}$	1.0 ± 2.2*	9.2 ± 1.3	8.2 ± 1.4
MVC	−1.3 ± 1.2*	−12.3 ± 1.2	−8.9 ± 1.3†
Voluntary muscle activation	−0.1 ± 1.0*	−6.9 ± 1.1	−3.7 ± 1.2†
M-wave amplitude	0.7 ± 2.7*	2.5 ± 2.0*	−7.8 ± 2.1

Changes in muscle function are expressed as a percent change from pre-exercise baseline. All exercise trials were performed for the same duration (10.6 ± 0.7 min) and at the same absolute workload (138 ± 14 W).Values are expressed as means ± SE. $Q_{tw,pot}$, potentiated single twitch; MRFD, maximal rate of force development; MRR, maximal rate of relaxation; $RT_{0.5}$, 1/2 relaxation time; MVC, maximal voluntary contraction force; M-wave, compound muscle action potential. Percent muscle activation is based on superimposed twitch technique. Various variables in acute and chronic hypoxia were, compared with baseline, altered significantly, 2.5 min after exercise ($P < 0.01$). *Not significantly different from pre-exercise baseline. †$P < 0.05$ vs. acute hypoxia, $n = 8$.

Compared with SL, C_aO_2 was approximately one-third lower and W_{insp}, ~34% higher during exercise in AH. These substantial alterations are known to contribute about equally to the exacerbated development of peripheral fatigue in AH (2). The impact of an acutely lowered C_aO_2 on muscle fatigability is mediated via the facilitating effects of the associated reduction in muscle O_2 delivery on the intramuscular accumulation of metabolites known to cause peripheral fatigue, i.e., hydrogen ion and inorganic phosphate (37, 61). The W_{insp}-induced exacerbation of peripheral fatigue results from the same intramuscular metabolic consequences associated with reductions in locomotor muscle O_2 delivery. However, in the case of the W_{insp}-related impairment in peripheral fatigue, the compromised O_2 delivery is the consequence of a sympathetically mediated impact on Q_L, secondary to the activation of the respiratory muscle metaboreflex (34). Taken together, the combined effects of a significantly reduced C_aO_2 and a higher W_{insp} has a profound impact on leg O_2 delivery and thus peripheral locomotor muscle fatigue (1).

Chronic hypoxia. Despite 2 wk of acclimatization to altitude, the rate of development of peripheral locomotor muscle fatigue was similar in AH and CH (Table 2 and Fig. 2). Somewhat conflicting data from earlier investigations suggest different mechanisms as a potential explanation of this finding. On the one hand, studies conducted by Reeves and colleagues (8, 64), following 2–3 wk at 4,300 m, report similar locomotor muscle O_2 delivery during submaximal endurance exercise in AH and CH. Given the critical dependency of the development of peripheral fatigue on muscle O_2 delivery, this similarity might explain the nearly identical levels of end-exercise locomotor muscle fatigue in AH and CH. On the other hand, experiments conducted at the same location as the present study (Mt. Chacaltaya, 5,260 m) have documented a significant improvement in leg muscle O_2 delivery from AH to CH, with the net

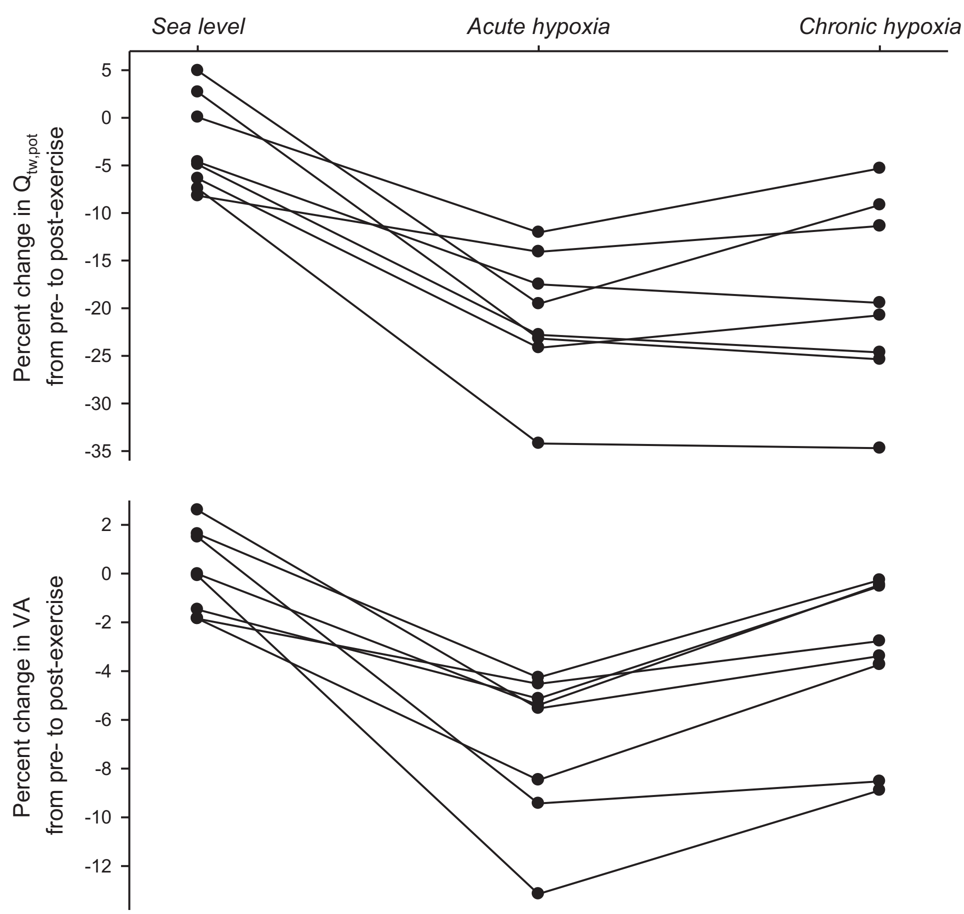

Fig. 2. Individual data illustrating the effects of constant-load bike exercise (138 ± 14 W; 10.6 ± 0.7 min) on potentiated quadriceps twitch force ($Q_{tw,pot}$; *top*) and voluntary muscle activation (VA; *bottom*) at sea level [SL; resting arterial oxygen (O_2) content: 19.3 ± 0.7 ml O_2/dl] and in AH (17.3 ± 0.5 ml O_2/dl) and chronic hypoxia (CH; 20.3 ± 1.3 ml O_2/dl).

effect of similar values during submaximal bike exercise at SL and in CH (13). It might be important to emphasize that these latter experiments involved a greater altitude (5,260 m vs. 4,300 m) and a 9–10 wk acclimatization period vs. only a 2–3 wk period, as in the experiments by Reeves and colleagues (8, 64), as well as the present study. Regardless, based on the findings from the earlier Chacaltaya experiments, it appears that the similar degrees of end-exercise fatigue in AH and CH in the present study (Fig. 2) might have occurred in the face of a significant difference in bulk muscle O_2 delivery, i.e., higher in CH vs. AH.

Q_L was not measured directly in the present study. However, changes in THb, a NIRS-derived variable, are thought to reflect changes in regional blood volume and potentially Q_L (24, 59). The previously documented similarity in resting Q_L at SL, in AH, and in CH (11, 12, 36, 49, 50) is a critical prerequisite when using THb as an estimate of potential differences in Q_L and O_2 delivery during exercise. Since C_aO_2 was comparable at SL and CH (see RESULTS), the same exercise-induced increase in THb (Fig. 3) suggests a similar degree of O_2 delivery in these conditions. Furthermore, the combination of a lower C_aO_2 in AH vs. CH (and SL; see RESULTS) plus the similar increase in THb during exercise (Fig. 3) insinuates a lower locomotor muscle O_2 delivery in AH vs. CH (and by extension, SL). Both of these observations might support earlier blood flow studies conducted at the same location as the present experiments (13) but might contradict others performed at a lower altitude (8, 64). However, NIRS findings obtained from skeletal muscle need to be interpreted with caution. A significant limitation associated with NIRS is that this measurement is confined to a finite location, and changes in THb might not be representative of the whole muscle. Indeed, significant blood flow heterogeneity has been documented previously in skeletal muscle (35). Whereas heterogeneity diminishes with higher exercise intensities and is not affected by hypoxia (36), the exact location of NIRS probe placement from day to day is a potential source of error. To minimize this risk, we had strict criteria regarding probe placement (see METHODS), and at least two investigators independently assured correct probe positioning before each experiment.

Assuming that the similar degrees of peripheral fatigue in AH vs. CH occurred in the face of a greater O_2 delivery in CH, other, rather disadvantageous adaptations associated with acclimatization must have outweighed this benefit. A potential candidate is the documented impairment in the capacity of skeletal muscle to extract O_2 in CH, i.e., a decreased capillary muscle O_2 conductance (41). This impact might, despite a similar O_2 delivery at SL and in CH, potentially lower extracellular PO_2 to or beyond a previously suggested critical value (~30 Torr) associated with exacerbated development of peripheral fatigue (55). Alternatively, the higher O_2 delivery in CH vs. AH (13), combined with the same degree of peripheral fatigue, might suggest that C_aO_2 and bulk O_2 delivery, per se, might not depict key determinants of the exaggerated fatigability in hypoxia. Important here is the fact that despite the normalized C_aO_2 and bulk O_2 delivery in CH, P_aO_2 only partially recovers with acclimatization and remains fairly low in CH. This could hint toward a key role of P_aO_2 in exacerbating the development of peripheral fatigue at altitude.

In CH, V_E was ~20% higher compared with AH. Given the substantially lower air density at 5,260 m (0.64 kg/m^3 vs. 1.18 kg/m^3 at 130 m, where AH experiments occurred), it could be argued that in terms of respiratory muscle work, the reduced density might balance the acclimatization-induced increase in V_E, with the net effect of a similar W_{insp} in CH and AH. However, W_{insp} was, similar to V_E, ~20% higher in CH vs. AH. This observation, per se, might suggest that the lower air density at altitude had no effect on the relationship between minute V_E and respiratory muscle work. However, it has been shown that bronchoconstriction, associated with severe hypoxia, increases the resistive component of respiratory work and offsets the theoretical benefit of a reduced air density (22). This results in a similar respiratory muscle work for a given V_E at altitude and at SL (18). Therefore, any increase in W_{insp}

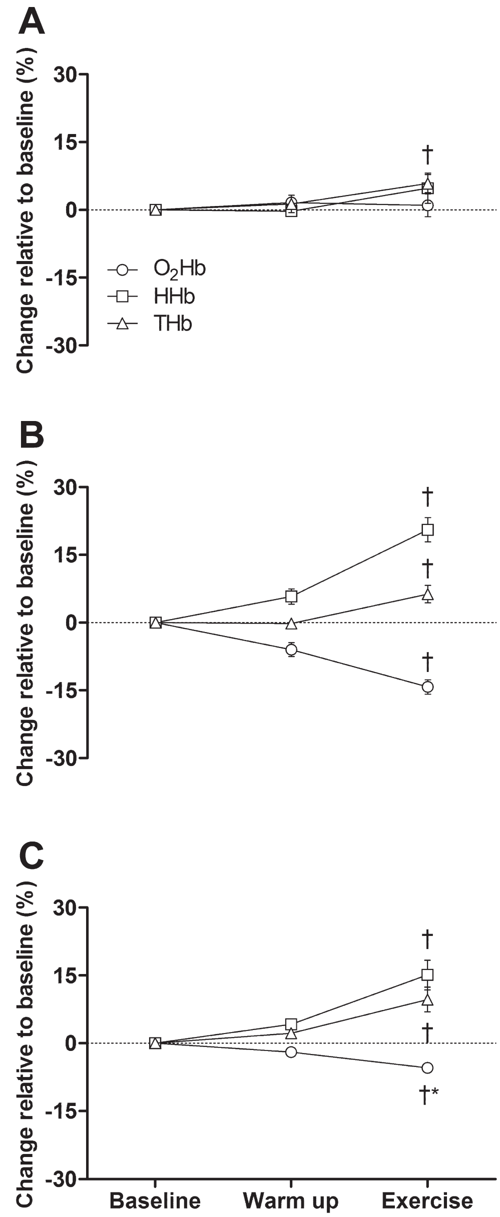

Fig. 3. Vastus lateralis oxygenation at resting baseline, during the final 30 s of a 3-min warm-up (28 W), and during the final 30 s of constant-load exercise (131 W) at SL (*A*), in AH (*B*), and in CH (*C*). †$P < 0.05$ vs. respective baseline; *$P < 0.05$ vs. AH, $n = 8$. O_2Hb, oxyhemoglobin; HHb, deoxyhemoglobin; THb, total hemoglobin.

observed in hypobaric CH is attributable to the exaggerated ventilatory response associated with altitude acclimatization.

The increase in minute V_E in the present study was mainly due to the increase in V_T; f_R was similar in both conditions. The higher V_T was achieved via reductions in EELV (Table 1), which is compared with increasing EILV to raise V_T, more economical, since higher lung volumes are associated with a reduced compliance (38). We therefore conclude that the 23% higher W_{insp} at the same workload in CH vs. AH resulted from the substantially higher V_E following acclimatization. Finally, this exaggerated W_{insp} likely aggravated the respiratory muscle metaboreflex and associated impact on leg vascular conductance (25) and presumably blunted exercise Q_L more in CH compared with AH.

In contrast to our findings, it was suggested previously that acclimatization to high altitude might eliminate the impact of AH on the rate of development of fatigue during single muscle exercise (adductor pollicis) and restore it to that observed at SL (28). However, submaximal, intermittent exercise, including a small muscle mass, does not maximally challenge O_2 delivery and use. Therefore, the observed positive effect could, at least in part, be explained by the use of the available reserve capacity. Specifically, various compensatory mechanisms, including increases in cardiac output and muscle O_2 delivery and extraction, could have reduced the hypoxia-induced impact on the development of fatigue. Such an effective compensation might not—or only to a much smaller degree–be possible during intense, whole-body exercise, performed close to a human's maximal circulatory and ventilatory capacity (14, 15).

CH had a significant impact on the effect of exercise on M-wave amplitude. Reductions in M-wave amplitude have been associated with decreases in sarcolemma excitability (19). The attenuated excitability results from reduced sarcolemma sodium (Na^+)-potassium (K^+)-ATPase activity (46) and can contribute to compromised muscle force output (21). Pre-exercise M-wave amplitudes (and $Q_{tw,pot}$) in our experiments were similar in all three conditions. This suggests that neither severe AH nor CH impairs sarcolemma Na^+-K^+-ATPase activity and membrane excitability of resting locomotor muscle. This confirms earlier findings (40); however, it contrasts with others (16) who report decreased resting M-wave amplitudes following 10 days of exposure to severe hypoxia (>4,300 m). Regardless, although M-wave amplitudes did not change from pre- to postexercise at SL and in AH, we observed, in contrast to Garner et al. (30), a significant exercise-induced decrease in CH (Table 2). AH has recently been shown to have no effect on exercise-induced changes in Na^+-K^+-ATPase activity, which explains the similar M-wave behavior in SL and AH (51). However, altitude acclimatization causes a downregulation of Na^+-K^+-ATPase pump concentration, and although this does not alter resting M-wave characteristics, it likely explains the exercise-induced decrease in M-wave amplitude observed in CH (20, 33).

The lower postexercise M-wave amplitude in CH indicates a failure of the motor nerve/sarcolemma to propagate evoked stimuli to the contractile apparatus and might have masked potential benefits of acclimatization on fatigue resistance. Put simply, postexercise twitch forces might have been larger in CH if M-waves had remained unchanged from pre-exercise. If so, this would have resulted in a smaller exercise-induced reduction in $Q_{tw,pot}$ in CH. Regardless, failure of neuromuscular transmission/sarcolemmal excitability contributes to reduced force output in response to a given central nervous activation and can therefore be considered a key determinant of the impaired fatigue resistance in CH.

Central Fatigue

Exercise in AH induced a substantial degree of central fatigue, which was attenuated by ~50% when the same trial was repeated in CH (Table 2). This significant improvement, associated with acclimatization, clearly contrasts with the absence of a beneficial effect of CH on peripheral fatigue, as described above. Since the development of central fatigue is highly sensitive to O_2 (1), we attribute this improvement to the effects of high-altitude acclimatization on O_2 availability within the brain. Specifically, the cerebral O_2 delivery index at the end of exercise in CH was improved from AH (Table 1) (65) and may explain the lower degree of central fatigue in CH vs. AH.

Despite the similar CBFv and a slightly higher brain O_2 delivery in CH vs. SL (Table 1), which agrees with earlier Chacaltaya studies using the Kety-Schmidt technique to measure CBF/O_2 delivery (44), exercise-induced central fatigue was greater in CH. Two considerations discussed previously might account for this observation. First, the significant degree of peripheral fatigue in CH (vs. no fatigue at SL) presumably facilitated central fatigue via increases in inhibitory neural feedback from locomotor muscle (mediated by *group III/IV* muscle afferents), which limit central motor drive (3, 6). Second, although C_aO_2 and brain O_2 delivery were similar/higher in CH vs. SL, the still substantially lower P_aO_2 might have contributed to the greater degree of central fatigue during exercise in this condition. Indeed, a low P_aO_2 was recently suggested to impair cerebral metabolism (48) and alterations in neurotransmitter turnover (23), and both of these factors have been linked to the development of central fatigue (17, 53).

Taken together, the current findings provide a global indication of the positive effects of altitude acclimatization on the development of central fatigue during exercise. However, we cannot comment on the specific sites of the central motor pathway involved or the relative contribution of C_aO_2 and P_aO_2 in mediating these beneficial adaptations.

Implications of Findings for Performance-Related Questions in CH

AH generally impairs endurance exercise performance (60). Prolonged exposure to hypoxia is known to recover some of this impairment (29, 52); however, SL performance is never matched at altitude. Our current findings indicate that the acclimatization-induced partial recovery of endurance performance occurs independent of any improvement of peripheral locomotor muscle fatigue from AH to CH. This insinuates that peripheral locomotor muscle fatigability, per se, does not contribute to the improvement of endurance performance observed from AH to CH. We therefore propose that the significantly attenuated central fatigue during exercise in severe CH likely accounts, at least in part, for the improvement of endurance performance associated with altitude acclimatization.

Mechanisms underlying the hypoxia-induced curtailment of central motor drive (i.e., increase in central fatigue) and endurance exercise performance have been documented previously to differ depending on the severity of arterial hypoxemia. Specifically, peripheral fatigue might depict the dominant determinant of central motor drive and thus the limiting factor above 70–75% S_pO_2. At more severe degrees of hypoxemia (<70% S_pO_2), central motor drive and endurance performance might primarily—but not exclusively—be determined/limited by central nervous system (CNS) hypoxia (5). Since peripheral fatigue did not change with acclimatization in the present study, but S_pO_2 increased from below to above the "threshold" described previously (5), reductions in central fatigue might be mediated mainly by improved arterial oxygenation and associated smaller influence of CNS hypoxia on central motor drive.

A recent Point:Counterpoint debate in this journal has focused on the potential existence/relevance of differences in physiological responses to exercise performed in normobaric vs. hypobaric hypoxia (43, 45). Since the present AH and CH experiments were performed in normobaric and hypobaric hypoxia, respectively, these potential differences, if indeed existent, might have influenced our findings.

Conclusion

AH exacerbates central and peripheral fatigue during endurance exercise. Our experiments indicate that acclimatization to high altitude significantly attenuates the development of central fatigue but does not improve the development of peripheral fatigue observed during whole-body endurance exercise in AH.

ACKNOWLEDGMENTS

This paper is part of a series, titled "AltitudeOmics," which together, represents a group of studies that explored the basic mechanisms controlling human acclimatization to hypoxia and its subsequent retention. Many people and organizations invested enormous time and resources to make AltitudeOmics a success. Foremost, the study was made possible by the tireless support, generosity, and tenacity of our research subjects. AltitudeOmics principal investigators were Colleen G. Julian, Andrew T. Lovering, Andrew W. Subudhi, and Robert C. Roach. We also thank Mr. Jui-lin Fan and Drs. Bengt Kayser and Nicolas Bourdillon (University of Geneva, Switzerland); Mr. Jim Davis, Mr. Jonathan Elliot, Dr. Steve Laurie, Ms. Julia Kern, Ms. Kara Beasley, and Mr. Henry Norris (University of Oregon); and Mr. Oghenero Evero (University of Colorado) for valuable technical assistance during data collection. In addition, we thank Drs. Lee Romer and Emma Ross for allocating nerve stimulation equipment from Brunel University and the University of Brighton (UK). Finally, we thank Dr. Jerry Dempsey for valuable advice and feedback on the manuscript.

GRANTS

Funding for AltitudeOmics was provided, in part, by grants from the Department of Defense (W81XWH-11-2-0040 Telemedicine and Advanced Technology Research Center to R. C. Roach and W81XWH-10-2-0114 to A. T. Lovering); the Cardiopulmonary & Respiratory Physiology Laboratory, University of Oregon; and the Altitude Research Center and the Charles S. Houston Endowed Professorship, Department of Emergency Medicine, School of Medicine, University of Colorado Denver. Additional funding for this study of fatigue in hypoxia was provided by the National Heart, Lung, and Blood Institute (HL-103786 and HL-116579 to M. Amann).

DISCLOSURES

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Author contributions: M.A., S.G., and A.W.S. conception and design of research; M.A., S.G., R.T., and A.W.S. performed experiments; M.A., S.G., and R.T. analyzed data; M.A., S.G., R.T., and A.W.S. interpreted results of experiments; M.A. and S.G. prepared figures; M.A. and A.W.S. drafted manuscript; M.A., S.G., R.T., A.W.S., A.T.L., and R.C.R. edited and revised manuscript; M.A., S.G., R.T., A.W.S., A.T.L., and R.C.R. approved final version of manuscript.

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Received Date : 04-Sep-2013

Revision Requested: 24-Oct-2013
Revised Date : 10-Dec-2013

Accepted Date : 17-Jan-2014

Article type : Regular Paper

AltitudeOmics: Exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatisation to high altitude

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Short Title: Central fatigue and acclimatisation to altitude

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Abstract

Aims: We asked whether acclimatisation to chronic hypoxia (CH) attenuates the level of supraspinal fatigue that is observed after locomotor exercise in acute hypoxia (AH). **Methods:** Seven recreationally-active participants performed identical bouts of constant-load cycling (131±39W, 10.1±1.4min) on three occasions: 1) in normoxia (N, P_IO_2, 147.1mmHg); 2) in AH (F_IO_2, 0.105; P_IO_2, 73.8mmHg); 3) after 14 days in CH (5,260m; P_IO_2, 75.7mmHg). Throughout trials, prefrontal-cortex tissue oxygenation and middle cerebral artery blood velocity (MCA_V) were assessed using near-infrared-spectroscopy and transcranial Doppler sonography. Pre- and post-exercise twitch responses to femoral nerve stimulation and transcranial magnetic stimulation were obtained to assess neuromuscular and corticospinal function. **Results:** In AH, prefrontal oxygenation declined at rest (Δ7±5%) and end-exercise (Δ26±13) ($P<0.01$); the degree of deoxygenation in AH was greater than N and CH ($P<0.05$). The cerebral O_2 delivery index ($MCA_v \times C_aO_2$) was 19±14% lower during the final minute of exercise in AH compared to N ($P=0.013$) and 20±12% lower compared to CH ($P=0.040$). Maximum voluntary and potentiated twitch force were decreased below baseline after exercise in AH and CH, but not N. Cortical voluntary activation decreased below baseline after exercise in AH (Δ11%, $P=0.014$), but not CH (Δ6%, $P=0.174$) or N (Δ4%, $P=0.298$). A twofold greater increase in motor evoked potential amplitude was evident after exercise in CH compared to AH and N. **Conclusion:** These data indicate that exacerbated supraspinal fatigue after exercise in AH is attenuated after 14 days of acclimatisation to altitude. The reduced development of supraspinal fatigue in CH may have been attributable to increased corticospinal excitability, consequent to an increased cerebral O_2 delivery.

Key words: adaptation, altitude, exercise, transcranial magnetic stimulation

Glossary

C_aO_2, arterial O_2 content; CSP, cortical silent period; ERT, estimated resting twitch; F_IO_2, fraction of inspired O_2; f_R, respiratory frequency; [Hb], haemoglobin concentration; MCA_V, middle cerebral artery blood velocity; MEP, motor evoked potential; M_{max}, maximum M-wave; MVC, maximum voluntary contraction; P_aO_2, partial pressure of arterial O_2; P_IO_2, partial pressure of inspired O_2; $Q_{tw,pot}$, potentiated quadriceps twitch force; rMT, resting motor threshold; SIT, superimposed twitch; S_pO_2, arterial O_2 saturation; TMS, transcranial magnetic stimulation; $\dot{V}CO_2$, carbon dioxide output; $\dot{V}_E$, minute ventilation; $\dot{V}O_2$, oxygen uptake; V_T, tidal volume.

Introduction

The mechanisms underpinning impairments in exercise performance in hypoxia are not fully understood, but multiple peripheral and central mechanisms of fatigue have been proposed (Amann and Calbet, 2008, Nybo and Rasmussen, 2007, Perrey and Rupp, 2009). The rate of development of peripheral fatigue is increased during intense locomotor exercise in acute hypoxia (Amann *et al*., 2006b, Goodall *et al*., 2012). This has been documented in numerous human studies as an increased decline in the force response to motor nerve stimulation after exercise and an increased rate of rise in electromyogram (EMG) signals during exercise (Amann and Calbet, 2008). Amann *et al*. (2006a) suggested that the accelerated development of peripheral fatigue and associated intramuscular metabolic changes in acute moderate hypoxia restricts central motor drive preventing excessive end-exercise locomotor muscle fatigue under conditions of attenuated arterial oxygenation. It was subsequently demonstrated that in acute severe hypoxia, peripheral fatigue becomes the less important variable and the primary limitation to exercise transfers to a hypoxia-sensitive central component of fatigue (Amann *et al*., 2007). Less is known about the mechanism(s) of fatigue during locomotor exercise in chronic hypoxia. We recently reported the accelerated development of peripheral fatigue after locomotor exercise in acute hypoxia to be similar after a period of acclimatisation (14 days) to high altitude; conversely, the level of central fatigue was attenuated (Amann *et al*., 2013). The measure of central fatigue, however, was determined using peripheral stimulation and the responsiveness of the brain-to-muscle pathway after a period of chronic hypoxia remains unknown.

Transcranial magnetic stimulation (TMS) has been used to specify the site of fatigue within the central nervous system in acute severe hypoxia (Goodall *et al*., 2012, Goodall *et al*., 2010). When TMS is delivered over the motor cortex during a maximal voluntary contraction (MVC), it is possible to detect a twitch-like increment in force in the active muscle. That is, despite maximal effort, motor cortical output at the time of stimulation is insufficient to drive the motoneurons maximally. An increase in this increment in force after exercise provides evidence of a reduced cortical voluntary activation, indicative of supraspinal fatigue (Gandevia *et al*., 1996, Todd *et al*., 2003). Further, EMG recordings in response to cortical stimuli (motor evoked potential [MEP]) can be monitored to assess changes in excitability of the brain to muscle pathway. Descending volleys evoked from cortical stimulation depend on the stimulus intensity and excitability of corticospinal cells, whereas responses in the muscle depend on transmission through relevant excitatory and inhibitory interneurons and excitability of the motoneuron pool (Taylor and Gandevia, 2001). Hypoxia affects neuronal function *in-vitro* (Nieber *et al*., 1999), however, acute hypoxia appears to have negligible effects on resting MEPs elicited by TMS (Goodall *et al*., 2010, Rupp *et al*., 2012, Szubski *et al*., 2006). A MEP evoked during muscular contraction is followed by an interval of EMG silence, the so-called cortical silent period (CSP). The initial phase of the CSP has been attributed to inhibitory spinal mechanisms (Inghilleri *et al*., 1993), whereas the later period (>100 ms) represents increased cortical inhibition (Chen *et al*., 1999, Inghilleri *et al*., 1993, Taylor and Gandevia, 2001). Szubski *et al*. (2006) found a shorter CSP in acute hypoxia, suggestive of a reduced corticospinal inhibition during the exercise.

Responsiveness of the corticospinal pathway and the associated development of central fatigue after locomotor exercise during periods of prolonged hypoxia have not been studied. A recent investigation found an increase in corticospinal excitability (increased resting MEP) after a period of prolonged acute hypoxia (Rupp *et al*., 2012); however, the mechanisms for this response and the associated effects upon the development of central fatigue during locomotor exercise have not been studied. We have recently related the development of supraspinal fatigue during exercise in severe acute hypoxia to a reduction in cerebral O_2 availability (Goodall *et al*., 2012). Acclimatisation to altitude not only brings about improvements in arterial oxygenation, but also improvements in cerebrovascular function (Ainslie and Ogoh, 2009, Lucas *et al*., 2011). It is unknown how haematologic (e.g., hemodynamic and cerebrovascular) adaptations might serve to impact corticospinal excitability and the development of supraspinal fatigue during locomotor exercise in chronic hypoxia. Accordingly, the aim of the present study was to assess corticospinal excitability and supraspinal fatigue after locomotor exercise in chronic hypoxia. We hypothesised that altered cerebrovascular and corticospinal responses after a period of acclimatisation to high altitude would reduce the severity of supraspinal fatigue compared to that observed in acute hypoxia.

Methods

Ethical Approval

All procedures conformed to the Declaration of Helsinki and were approved by the Universities of Colorado Denver, Oregon and Utah Institutional Review Boards and the US Department of Defense Human Research Protection Office.

Participants

This study was conducted as part of the AltitudeOmics project examining the integrative physiology of human responses to hypoxia (Subudhi *et al.* under review at PLoSOne). After written informed consent, seven (five male) recreationally active sea level habitants participated in the study (mean ± SD age, 21 ± 1 yr; stature, 1.78 ± 0.10 m; body mass, 69 ± 11 kg; maximum O_2 uptake [$\dot{V}O_{2max}$], 46.4 ± 8.2 ml·kg^{-1}·min^{-1} [participant IDs: 1,2,3,5,6,7,10]). The participants were non-smokers, free from cardiorespiratory disease, born and raised at <1500 m, and had not travelled to elevations >1000 m in the 3 months prior to investigation. Participants arrived at the laboratory in a rested and fully hydrated state, at least 3 h postprandial, and avoided strenuous exercise in the 48 h preceding each trial. They also refrained from caffeine for 12 h before each test, while alcohol and prophylactic altitude medication were prohibited for the entire duration of the investigation. All of the subjects participated in a companion study investigating the acclimatisation-induced effects on peripheral measures of neuromuscular fatigue (Amann *et al*., 2013); while the data were obtained from the same protocol described below, the primary TMS and cerebral oxygenation related outcome measures in the current study do no overlap with previous analyses.

Experimental design

Participants completed a preliminary trial and three experimental trials. Each trial was conducted at the same time of day, and separated by at least 5 d during a 12 wk period. During the preliminary trial, participants were thoroughly familiarized with the methods used to assess neuromuscular function and performed a maximal incremental exercise test in normoxia for the determination of $\dot{V}O_{2max}$ and peak workload (W_{peak}); further maximal incremental tests were performed in AH and CH (Subudhi *et al*. under review at PLoSOne). During the experimental trials, participants performed constant-load exercise at a workload equal to 50% W_{peak} obtained in the preliminary trial: 1) to the limit of tolerance in acute normobaric hypoxia (AH: F_IO_2 = 0.105; Eugene, Oregon, barometric pressure [BP] = 750 ± 2 mmHg; P_IO_2 = 73.8 ± 0.2 mmHg); 2) for the same absolute intensity and duration as in trial 1, but in normoxia (N: Eugene, Oregon, BP = 750 ± 2 mmHg; P_IO_2 = 147.1 ± 0.5 mmHg); and 3) for the same absolute intensity and duration as in trial 1, but after 14 d at 5,260 m above sea level (CH: Mt. Chacaltaya, Bolivia, BP = 409 ± 1 mmHg; P_IO_2 = 75.7 ± 0.1 mmHg). Participants were flown to La Paz, Bolivia where they spent two nights at low altitude (Coroico, 1,525 m), before being driven to the Chacaltaya Research Station at 5,260 m. Before and within 2.5 min after each exercise trial, twitch responses to supramaximal femoral nerve stimulation and TMS were obtained to assess fatigue. During AH, the post-exercise measurements were made while participants continued to breathe the hypoxic gas. Cerebrovascular, cardiorespiratory and perceptual responses, as well as EMG activity of the vastus lateralis (VL), were assessed throughout each trial.

Force and EMG recordings

Knee-extensor force during voluntary and evoked contractions was measured using a calibrated load cell (Tedea, Basingstoke, UK). The load cell was fixed to a custom-built chair and connected to a non-compliant cuff attached around the participant's right leg just superior to the right ankle. Participants sat upright in the chair with the hips and knees at 90° of flexion. EMG activity was recorded from the VL and biceps femoris (BF). Surface electrodes were placed 2 cm apart over the muscle bellies and a reference electrode was placed over the patella. The electrodes were used to record the compound muscle action potential (M-wave) elicited by electrical stimulation of the femoral nerve and the MEP elicited by TMS. Signals were amplified (gain 1000; Force: custom-built bridge amplifier; EMG: PowerLab 26T, ADInstruments Inc, Oxfordshire, UK), band-pass filtered (EMG only: 20-2000 Hz), digitised (4 kHz; PowerLab 26T, ADInstruments Inc), acquired and later analysed (LabChart v7.0, ADInstruments Inc).

Neuromuscular function

Force and EMG variables were assessed before and immediately after each exercise trial. Prior to each trial, MVC force was determined from three, 3 s contractions. Femoral nerve stimulation was delivered at rest ~2 s after the MVC to determine the potentiated quadriceps twitch force ($Q_{tw,pot}$). TMS was delivered during brief (~5 s) maximal and submaximal voluntary contractions for the determination of cortical voluntary activation. Each set of contractions comprised 100, 75, and 50% MVC efforts separated by ~5 s of rest. The contraction sets were repeated three times, with 15 s between each set. Visual feedback of the target force was provided via a computer monitor.

Femoral nerve stimulation

Single electrical stimuli (200 μs) were delivered to the right femoral nerve via surface electrodes (CF3200, Nidd Valley Medical Ltd, North Yorkshire, UK) and a constant-current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). The cathode was positioned over the nerve high in the femoral triangle; the anode was placed midway between the greater trochanter and the iliac crest. The site of stimulation that produced the largest resting twitch amplitude and M-wave (M_{max}) was located. Single stimuli were delivered beginning at 100 mA and increasing by 20 mA until plateaus occurred in twitch amplitude and M_{max}. Supramaximal stimulation was ensured by increasing the final intensity by 30% (mean current 253 ± 60 mA).

Transcranial magnetic stimulation

TMS was delivered via a concave double cone coil (110 mm diameter; maximum output 1.4 T) powered by a mono-pulse magnetic stimulator (Magstim 200, The Magstim Company Ltd, Whitland, UK). The coil was held over the vertex to preferentially stimulate the left hemisphere (postero-anterior intracranial current flow), and was placed in an optimal position to elicit a large MEP in the VL and a small MEP in the antagonist (BF). The optimal coil position was marked on the scalp with indelible ink to ensure reproducibility of the stimulation. Resting motor threshold (rMT) was determined at the beginning of each experimental trial. Briefly, TMS was first delivered with the coil placed over the optimal site of stimulation at a sub-threshold intensity of 35% maximum stimulator output. Stimulus intensity was then increased in 5% steps until consistent motor evoked potentials (MEPs) with peak-to-peak amplitudes of more than 50 μV were evoked. Thereafter, stimulus intensity was reduced in 1% steps until an intensity was reached that elicited an MEP of at least 50 μV in 5 out of 10 trials (Groppa *et al.*, 2012). The stimulation intensity that elicited rMT was increased by 30%; thus, the experimental stimulation intensity was 130% of rMT. This stimulation intensity elicited a large MEP in the VL (area between 60 and 100% of M_{max} during knee-extensor contractions ≥50% MVC; Figure 1); indicating the TMS stimulus activated a high proportion of knee extensor motor units, while causing only a small MEP in the BF (amplitude <20% of MEP during knee-extensor contractions).

Constant-load exercise

Participants sat on an electromagnetically-braked cycle ergometer (Velotron Dynafit Pro, Racermate, Seattle, WA) while baseline cardiorespiratory and cerebrovascular data were collected for 3 min. The participants warmed-up for 5 min at 10% W_{peak} (26 ± 8 W) before the workload was increased to 50% normoxic W_{peak} (131 ± 39 W). This intensity was chosen to maximise the tolerable duration of exercise in the hypoxic conditions. The participants remained seated throughout exercise and maintained a target pedal cadence equivalent to that chosen during the incremental exercise test (88 ± 3 rpm). Task-failure was reached when cadence dropped below 60% of the target rpm for >5 s. Constant load exercise was performed firstly in AH; the achieved time (10.1 ± 1.4 min) was then replicated in N and CH.

Tissue oxygenation and cerebrovascular responses

Cerebral oxygenation was assessed using a multi-channel NIRS instrument (Oxymon III, Artinis) (Subudhi *et al*., 2009, Subudhi *et al*., 2011). Changes in oxygenated , deoxygenated and total cerebral haeme concentrations (μM) were expressed relative to the resting baseline recorded in each experimental condition. Arterial oxygen saturation was estimated using forehead pulse oximetry (S_pO_2; Model N-595, Nellcor, Pleasonton, CA). Excellent agreement between the pulse oximeter and arterial O_2 saturation across the range of values in the present study has been published (Romer *et al*., 2007). Hemoglobin concentration [Hb] was measured (OSM-3, Radiometer, Copenhagen, Denmark) in resting arterial blood samples. Samples were collected during the primary physiological protocols at sea level (2-4 d prior to the first exercise trial in the present study) and on the 16th day at 5,260 m (2 d following the constant load exercise trial in the present study) (Subudhi *et al*. under review at PLoSOne). Arterial O_2 content (C_aO_2) was estimated using the equation: ([Hb] × 1.39 × S_pO_2 / 100). Resting [Hb] in combination with the measured S_pO_2 during the exercise protocol were used to obtain C_ao_2 throughout exercise in all conditions. Blood velocity in the left middle cerebral artery (MCA_v) was determined using transcranial Doppler (Spencer Technologies, Seattle, WA). The custom-made NIRS headset was modified to hold a 2 MHz probe positioned over the left temporal window. Measurements were optimised at an average penetration depth of 50 ± 3 mm. An index of cerebral O_2 delivery was calculated as the product of MCA_v and C_aO_2. It was assumed that changes in MCA_V would reflect changes in cerebral blood flow based on evidence that the middle cerebral artery diameter changes minimally in response to hypoxia and hypocapnia (Poulin and Robbins, 1996).

Cardiorespiratory and perceptual responses

Ventilatory and pulmonary gas exchange indices were assessed using an online system (in AH & N Medical Graphics PFX, St. Paul, MN, USA; & in CH Oxigraf O_2cap, Mountain View, CA, USA). Heart rate was identified from the peak MCA_v envelopes. Ratings of perceived exertion for dyspnea and limb discomfort were obtained using the CR10 scale at baseline and every minute throughout exercise (Borg, 1982). In CH, symptoms of acute mountain sickness were assessed on the day of a trial using the Lake Louise Score (Roach *et al*., 1993).

Data analysis

Cortical voluntary activation was assessed by measuring the force responses to motor-cortex stimulation during submaximal and maximal contractions. Corticospinal excitability increases during voluntary contraction (Rothwell *et al.*, 1991); thus, we estimated the amplitude of the resting twitch evoked by TMS (ERT; Goodall *et al.*, 2009, Sidhu *et al.*, 2009a). Cortical voluntary activation (%) was subsequently quantified using the equation: (1 – [SIT / ERT] × 100).

The peak-to-peak amplitude and area of evoked MEPs and M_{max} were measured offline. To ensure the motor cortex stimulus activated a high proportion of the knee-extensor motor units, the area of vastus lateralis MEP was normalised to that of M_{max} elicited during the MVC at the beginning of each trial (Taylor *et al.*, 1999) (Figure 1). The duration of the CSP evoked by TMS during MVC was quantified as the duration from stimulation to the continuous resumption of post-stimulus EMG exceeding ± 2 SD of pre-stimulus EMG (>50 ms prior to stimulus). VL EMG signals during exercise were rectified and smoothed (15 ms), then quantified as the mean integrated area during each cycle revolution and averaged over each minute of exercise. A computer algorithm identified the onset and offset of activity where the rectified EMG signals deviated >2 SD from baseline for >100 ms.

Reliability coefficients

On a separate day, the responses to TMS, femoral nerve stimulation and MVC were repeated twice in all participants. The two assessment procedures were separated by a 2 min walk followed by 5 min of rest. Coefficient of variation (CV) and intraclass correlation coefficient (ICC) were calculated to evaluate test-retest reliability. All correlations were statistically significant and indicated, in combination with the CVs, a high level of reproducibility: cortical voluntary activation, CV = 1.4%, ICC = 0.82; CSP, CV = 7.1%, ICC = 0.93; ERT, CV = 10.2%, ICC = 0.84; MEP/M_{max}, CV = 9.6%, ICC = 0.66; M_{max}, CV = 11.4%, ICC = 0.98; 100% MVC MEP, CV = 14.1%, ICC = 0.96; 75% MVC MEP, CV = 10.2%, ICC = 0.98; 50% MVC MEP, CV = 7.2%, ICC = 0.99; MVC, CV = 4.7%, ICC = 0.94; $Q_{tw,pot}$, CV = 4.8%, ICC = 0.97.

Statistical analysis

Data are presented as means ± SD in the text and means ± SE in the figures. A 3 × 2 repeated measures ANOVA on condition (3 [AH, N, CH]) and time (2 [pre, post]) was used to test for within-group differences. When ANOVA revealed significant interactions, post-hoc comparisons were made using the least significant differences test. Statistical significance was set at $P < 0.05$. All analyses were conducted using SPSS (v19, IBM Corporation, New York, USA).

Results

Exercise responses

The exercise workload was 131 ± 39 W (50% N W_{peak}), which equated to 83% W_{peak} in AH and 74% W_{peak} in CH. Cerebral oxygenation data are shown in Figure 2. During N, oxyhaemoglobin was unchanged from baseline to warm up and total haemoglobin was increased during the final minute of exercise (P = 0.658 and 0.007, respectively). During AH, deoxygenated haemoglobin increased from baseline to warm up (P = 0.006); this response was exaggerated towards end exercise (P < 0.001). During CH, deoxygenated haemoglobin increased at end exercise (P = 0.015) in line with increased total haemoglobin (P = 0.043). Overall, these results demonstrate that the degree of cerebral deoxygenation (Δ deoxygenated haemoglobin) in AH was greater than that observed in N and CH (P < 0.05).

S_pO_2 and MCA_v data are shown in Figure 3. Acute exposure to hypoxia decreased S_pO_2 at rest (Δ7 ± 4%; P = 0.009) and during the final minute of exercise (Δ34 ± 10%; P < 0.001). Resting S_pO_2 in CH was 85 ± 2% (P < 0.001 vs. N; P = 0.330 vs. AH), and in the final minute of exercise had fallen to 78 ± 5% (P < 0.001 vs. N; P = 0.002 vs. AH). No changes in S_pO_2 were apparent in N (P > 0.702). Resting MCA_v did not differ between conditions at baseline (pooled average, 54 ± 9 $cm \cdot s^{-1}$; P = 0.544). MCA_v did not increase from rest at any time point in N (P > 0.108). MCA_v increased from rest to the final minute of exercise in AH (40 ± 15%; P < 0.001) and CH (25 ± 14%; P = 0.016), but did not differ between conditions (Figure 3).

Hemoglobin concentration was 1.42 ± 0.03 $g \cdot L^{-1}$ in N and 1.63 ± 0.31 $g \cdot L^{-1}$ in CH (P = 0.005). Resting P_aO_2 was reduced in AH compared to N (39.1 ± 4.8 vs. 103.3 ± 8.7 mmHg, P < 0.001), was increased in CH relative to AH (58.8 ± 3.2 mmHg, P < 0.001), but was still lower than N (P < 0.001). C_aO_2 was lower at rest in AH vs. N (19.8 ± 1.9 vs. 21.5 ± 2.9 $ml \cdot dl^{-1}$; P = 0.013); during the final minute of exercise C_aO_2 in AH was 36 ± 8% lower than N (P < 0.001) and 22 ± 9% lower than in CH (P = 0.001). C_aO_2 was lower at rest in CH vs. N (19.4 ± 2.6 vs. 21.5 ± 2.9 $ml \cdot dl^{-1}$; P < 0.001) and during the final minute of exercise (17.6 ± 2.9 vs. 21.2 ± 2.9 $ml \cdot dl^{-1}$; P = 0.725). Consequently, cerebral O_2 delivery index ($MCA_v \times C_aO_2$) was 19 ± 14% lower during the final minute of exercise in AH compared to N (P = 0.013) and 20 ± 12% lower compared to CH (P = 0.040). No differences were evident between N and CH at rest (P = 0.783) or during the final minute of exercise (P = 0.797) (Figure 3).

Cardiorespiratory data are shown in Table 1. Respiratory frequency and minute ventilation ($\dot{V}_E$) rose substantially over time in all conditions. $\dot{V}_E/\dot{V}CO_2$ during the final minute of exercise in AH and CH was approximately twofold greater than in N (P < 0.001); $\dot{V}_E/\dot{V}CO_2$ during the final minute of exercise was 28% higher in CH compared to AH (P < 0.001). During the final minute of exercise, whole body $\dot{V}O_2$ was not different across the three conditions (P = 0.411). Dyspnea and limb discomfort at end-exercise were higher in AH compared to N (P < 0.001 and P = 0.048, respectively), but were not different compared to CH (P = 0.714 and 0.549, respectively). Integrated EMG activity at end exercise was higher in AH compared to N (32%; P = 0.029), but not CH (16%; P = 0.303). There were no reported symptoms of acute mountain sickness during CH.

Pre- and post-exercise responses

Peripheral and central measures of excitability are shown in Table 2.

Neuromuscular responses

MVC did not differ between conditions at baseline (AH, 392 ± 77 N; N, 386 ± 90 N; CH, 376 ± 39 N; P = 0.942). MVC was reduced post-exercise in AH (339 ± 77 N, P = 0.011) and CH (346 ± 93 N, P = 0.032), but not N (387 ± 87 N, P = 0.684). The reductions in MVC were not different between conditions (P ≥ 0.119). $Q_{tw,pot}$ did not differ between conditions at baseline (AH, 107 ± 13 N; N, 105 ± 12 N; CH, 110 ± 16 N; P = 0.752). $Q_{tw,pot}$ was reduced post-exercise in AH (84 ± 14 N, P = 0.005) and CH (90 ± 18 N, P = 0.011), but not N (102 ± 12 N, P = 0.692). On average, resting M_{max} in CH displayed a twofold increase compared to AH and N (P < 0.019); however, the change in M_{max} during MVC was not statistically significant (P > 0.058). Neither measure of M_{max} changed pre- to post-exercise in any condition (P ≥ 0.610). Pooled across conditions, pre-exercise ERT (mean r^2 = 0.95) was 70% of the pre-exercise $Q_{tw,pot}$ and did not differ between conditions (mean ERT 75 ± 25 N; P = 0.811). Post-exercise ERT was reduced in AH (52 ± 27 N, P = 0.049), but was unchanged in N and CH (P ≥ 0.107).

Corticomotor responses

rMT in AH, N and CH was 54 ± 5, 53 ± 3 and 51 ± 6% maximum stimulator output (P = 0.276), respectively. During CH, resting MEP amplitude was twofold greater compared to AH (P = 0.014) and N (P = 0.014). Exercise elicited a reduction in resting MEP amplitude in CH (P = 0.022), but not AH (P = 0.346) or N (P = 0.369). MEPs evoked during brief knee extensor contractions at 100, 75 and 50% MVC pre-exercise were higher in CH compared to AH (P < 0.020) and N (P < 0.030) (see also Figure

4). MEPs evoked during the brief knee-extensor contractions (50-100% MVC) post-exercise were not significantly different from pre-exercise values in any condition. MEP amplitude, however, was higher post-exercise during CH compared to AH (50% MVC, P = 0.018; 75% MVC, P = 0.030) and N (50% MVC, P = 0.034). The MEP/M_{max} ratio increased for within contraction responses during CH (vs. AH 50 and 75% MVC; P ≤ 0.014 and N 50% MVC; P = 0.019) (Table 2). The CSP did not differ between conditions pre-exercise (pooled average, 186 ± 47 ms; P = 0.880) or post-exercise (pooled average, 185 ± 50 ms; P = 0.760). Baseline cortical voluntary activation did not differ between conditions (AH, 93 ± 5%; N, 97 ± 3%; CH, 93 ± 6%; P = 0.310) (Figure 5). Cortical voluntary activation was reduced post-exercise in AH (Δ11%, P = 0.014), but not in N (Δ4%, P = 0.298) or CH (Δ6%, P = 0.174); the decrease in AH was greater compared to N (P = 0.022) (Figure 5).

Discussion

The aim of the present study was to assess corticospinal excitability and supraspinal fatigue after locomotor exercise in chronic hypoxia. The main finding was that exercise-induced supraspinal fatigue, as quantified via changes in cortical voluntary activation, was attenuated after two weeks of acclimatisation to high altitude whereas it was exacerbated in AH vs. N. Importantly, the diminished level of central fatigue in CH occurred in parallel with improvements in cerebral haemodynamics and arterial oxygenation (increased C_aO_2 and S_pO_2) brought about by the two weeks at altitude. Moreover, the attenuated development of central fatigue occurred in line with a substantial increase in corticospinal excitability. This latter finding suggests that a period of acclimatisation modifies the integrity of the corticospinal tract. We confirm our hypothesis that acclimatisation to altitude reduces the level of exercise-induced central fatigue and that this is attributable, at least in part, to an increased overall excitability of the brain to muscle pathway.

Supraspinal Fatigue

A key aim of the present study was to determine the effect of acclimatisation on the development of central fatigue assessed after exercise. We hypothesised that improvements in cerebral oxygenation known to occur after a prolonged stay at altitude would bring about positive modifications on the development of central fatigue. We show that the development of supraspinal fatigue during locomotor exercise is recovered after 2 weeks at high altitude and similar to that observed in normoxia. Thus, the adaptive processes that take place during acclimatisation to high altitude seemingly protect healthy humans against the development of supraspinal fatigue.

Corticomotor responses

The present study found no change in corticospinal excitability (Δ resting MEP) in AH, a finding which is in line with literature utilising varying severities of hypoxia (F_IO_2 = 0.14 – 0.10; resting S_pO_2 = 93 - 74%) for as little as 10 min to 1 h (Goodall *et al.*, 2010, Rupp *et al.*, 2012, Millet *et al.*, 2012). However, Szubski *et al.* (2006) reported increased corticospinal excitability, expressed as a reduced rMT (not ΔMEP), after ~30 min of breathing hypoxic air (F_IO_2 = 0.12; resting S_pO_2 = 75%). Moreover, the present study found a twofold increase in corticospinal excitability after 14 d acclimatisation to severe altitude (5,260 m, equivalent to F_IO_2 0.105; resting S_pO_2 = 91 ± 2%) with accompanying increases in the MEP/M_{max} ratio, suggesting that the increases in MEP size were due to adaptive mechanisms within spinal and/or supraspinal sites. Similarly, Rupp *et al*. (2012) found a 26% increase in corticospinal excitability (ΔMEP amplitude) after 3 h of exposure to normobaric hypoxia (F_IO_2 = 0.12; resting S_pO_2 = 86%), demonstrating a time-dependent, hypoxia-induced modification in the brain-to-muscle pathway. Thus, a prolonged stay at altitude modifies the integrity of the corticospinal pathway which may contribute to reduce the level of central fatigue; however, a duration-dependent adaptation cannot yet be established with certainty.

TMS over the motor cortex preferentially activates corticospinal neurons trans-synaptically through excitatory interneurons and corticocortical axons (Di Lazzaro *et al*., 1998). The response to TMS critically depends on membrane excitability of motor cortical neurons and ion-channel function (Boroojerdi *et al*., 2001, Rothwell *et al*., 1991). *In vitro* investigations using isolated cerebral neurons from rats demonstrate that ion-channel function is affected by O_2 availability and that neuronal hyper-excitability is the consequence of chronic hypoxia (Donnelly *et al.*, 1992). A heightened neural response is necessary to maintain membrane integrity and ionic homeostasis that occur from a period of insufficient metabolic activity (Nieber *et al*., 1999). Thus, the twofold increase in MEP observed in the present study might be due to facilitated cortical neurons acting to restore the loss of neuronal activity associated with a prolonged exposure to altitude. Additionally, an increased level of muscle sympathetic nerve activity (peroneal microneurography) has been reported during a prolonged stay at the same altitude as in the present study (Hansen and Sander, 2003). That study showed a significant increase in muscle sympathetic nerve activity just 3 days after exposure to high altitude, suggesting that the prolonged stay induced a striking and long-lasting sympathetic over-activity. More recently, Buharin *et al*. (2013) found that a transient increase in sympathetic nerve activity (induced via lower body negative pressure) enhances corticospinal excitability as identified using TMS. The mechanism responsible for the increase in corticospinal excitability was postulated to be due to an elevated concentration of noradrenaline, a monoamine that is known to increase exponentially during sustained periods at altitudes exceeding 4,000 m (Cunningham *et al*., 1965, Mazzeo *et al*., 1994). Thus, the increased corticospinal excitability observed following 2 weeks of acclimatisation in the present study might be attributable, at least in part, to a heightened sympathetic nerve activity and associated increases in corticospinal excitability as well as hyper-excitable cerebral neurons. The increased corticospinal excitability in this investigation occurred in line with no symptoms of mountain sickness, a finding that opposes that of Miscio *et al*. (2009). Miscio *et al*. (2009) found that exposure to high altitude changes cortical excitability by affecting both inhibitory and excitatory circuits and that this is reflected in acute mountain sickness symptoms. This conclusion was based on a group of participants who resided at 4,554 m for only 3-5 days, a time frame in which acute mountain sickness is said to be most prominent (Hackett and Roach, 2001) and much shorter than the present study.

Despite substantial differences in end-exercise peripheral fatigue, CSP duration immediately after exercise (i.e., pre-to post-exercise change) was similar in all conditions. This suggests that locomotor exercise in N, AH and CH does not influence intracortical inhibition. These findings are in agreement with investigations using locomotor exercise in N and AH (Goodall *et al*., 2012, Sidhu *et al*., 2009b). However, Oliviero *et al*. (2002) reported decreased intracortical inhibition and CSP duration in chronic hypoxemic patients with COPD. These changes, mediated by cerebral GABA receptors, were reversed after 3-4 months of O_2 therapy, demonstrating that the changes were O_2 sensitive. However, factors other than chronic hypoxaemia might influence intracortical inhibition in patients with COPD making it difficult to quantify the influence that chronic hypoxaemia has on cortical inhibition.

On balance, we judge the increased corticospinal excitability in CH noted in the present study to be the result of adaptations in ion-channel function and elevations in circulating catecholamines serving to facilitate neurotransmission rather than mechanisms related to intracortical inhibition (Buharin *et al*., 2013, Nieber *et al*., 1999, Palange, 1998).

Hematological and cerebrovascular responses

Upon initial exposure to high altitude, acute hypoxia dilates cerebral arterioles thereby overriding the vasoconstrictive effect of hyperventilation-associated hypocapnia (Iwasaki *et al*., 2011). During a prolonged stay at altitude, hypocapnia further develops and arterial hypoxaemia is ameliorated, as reflected by increases in arterial [Hb], PO_2 and O_2 saturation (Figure 3). Furthermore, the increase in P_aO_2 and further decrease in P_aCO_2 with acclimatisation causes relative vasoconstriction reducing CBF down to SL values (Subudhi *et al*. 2013). We estimated an index of cerebral O_2 delivery using the product of MCA_v and C_aO_2. Our data demonstrate a reduced cerebral O_2 delivery index during exercise in AH compared to N; however, an improved cerebral O_2 delivery index was evident after two weeks of acclimatisation (Figure 3). The data in AH support a relationship between cerebral O_2 delivery and supraspinal fatigue (Goodall *et al*., 2012). The calculation of C_aO_2 during exercise from resting [Hb] should be interpreted with caution as a hemoconcentration could have impacted this measure. At sea level, the hemoconcentration accompanying maximal exercise for approximately 10 min is counterbalanced by the concomitant exercise-induced arterial hypoxemia with the net effect of similar C_aO_2 at rest and during exercise (Amman *et al*., 2006a). At altitude, despite significant hemoconcentration, C_aO_2 actually falls from rest to submaximal/maximal exercise by 10-25% (Calbet *et al*., 2003). This would suggest that exercise C_aO_2 calculations, based on a resting C_aO_2 measure, might actually overestimate C_aO_2 measured during exercise at altitude. Furthermore, we assumed that MCA diameter would remain constant in hypoxia (Poulin and Robbins, 1996, Serrador *et al*., 2000). While there is evidence of MCA dilatation at rest in hypoxia (Willie *et al*., 2012, Wilson *et al*., 2011), there is currently no evidence of MCA dilatation during intense exercise accompanied with substantial exercise-induced hyperventilation and associated hypocapnia. We acknowledge, however, that our measurements of blood velocity (rather than flow) must be interpreted with caution.

We found acclimatisation-induced increases in O_2 saturation and content (Figure 3). Furthermore, arterial O_2 tension increased from AH to CH (~39 mmHg to ~59 mmHg). Subudhi *et al*. (2013) has shown resting cerebral O_2 delivery to be maintained at levels observed in N during AH and CH, although it is presumed that the delivery of O_2 to the mitochondria within the parenchyma will be reduced because the driving gradient for diffusion from capillary to tissue is the PO_2 difference between capillary and tissue (Xu and Lamanna, 2006). The tissue PO_2 would be close to zero; thus, the driving force is essentially the P_aO_2. In the present study the P_aO_2 increased in line with acclimatisation, thereby improving the gradient for diffusion and perhaps restoring brain tissue O_2 tension to pre-hypoxic levels (Dunn *et al*., 2000). Thus, we postulate that the lack of central fatigue in chronic hypoxia may be related to increases in brain tissue O_2 tension. However, the link between increases in P_aO_2 and C_aO_2 and the reduction in central fatigue that occurs after a period of acclimatisation warrants further investigation.

Technical Considerations

Exercising in a hypobaric environment was not feasible for the trials in AH. Thus, the two modes of hypoxia (normobaric [AH] vs. hypobaric [CH]) differed. The literature concerning the responses in normobaric and hypobaric hypoxia is equivocal and readers are directed elsewhere to a point:counterpoint debate (Girard *et al*., 2012). Briefly, it was proposed that evidence is growing, suggestive that hypobaric hypoxia affects responses (ventilation, fluid balance, acute mountain sickness and performance) to a greater extent than normobaric hypoxia (Girard *et al*., 2012). However, this argument was opposed by the fact that in terms of O_2 sensing, hypobaric hypoxia does not induce different responses compared to normobaric hypoxia (Mounier and Brugniaux, 2012). Moreover, it is unknown how any such differences which might exist between hypobaric and normobaric hypoxia may affect indices of exercise-induced fatigue. We set the F_IO_2 (0.105) at sea level to obtain the same P_IO_2 (~74 mmHg) that was expected at the subsequent altitude in Bolivia (5,260 m).

In line with other investigations that have measured exercise-induced fatigue of the knee extensors (Goodall *et al*., 2012, Goodall *et al*., 2010, Sidhu *et al*., 2009b, Rossman *et al*., 2013), measurements were made within 2.5 min after exercise termination. Corticospinal excitability associated with maximal single muscle contractions recovers within 1 min post-exercise (Taylor *et al*., 1999). Thus, the present experimental design, utilising whole body exercise, might not have captured all elements of central fatigue. However, the methods and time to assess fatigue after exercise in all three conditions were identical and even though our measurements were made more than 1 min post-exercise, significant differences were observed, testifying to the strength of our data.

Conclusion

The novel finding was that supraspinal fatigue, present after exercise in acute hypoxia, was attenuated after a period of acclimatisation to high altitude. Importantly, the reduced development of central fatigue in chronic hypoxia occurred in parallel with an increase in the excitability of the brain to muscle pathway consequent to an increased cerebral O_2 delivery. The attenuated rate of development of central fatigue in chronic hypoxia might explain, at least in part, the improvements in locomotor exercise performance that are commonly observed after acclimatisation to high altitude.

Author Contributions

SG, RT, and MA contributed to conception and design of the experiments, data collection, data analysis, data interpretation, manuscript drafting and editorial process. ER contributed to conception and design of the experiments, data interpretation and manuscript revision. AL contributed to data collection. LR contributed to conception and design of the experiments, data interpretation, manuscript drafting and revision. AL, AS and RR conceived, designed and executed the AltitudeOmics study of which the present study was a part, and contributed to manuscript revision. AS also contributed to data collection and data interpretation. All authors approved the final version of the manuscript.

Acknowledgments

This paper is part of a series of papers, titled "AltitudeOmics", which together represent a group of studies that explored the basic mechanisms controlling human acclimatisation to hypoxia and its subsequent retention. Many people and organizations invested enormous time and resources to make this project a success. Foremost, the study was made possible by the tireless support, generosity and tenacity of our research subjects. AltitudeOmics principal investigators were Colleen G. Julian, Andrew T. Lovering, Andrew W. Subudhi and Robert C. Roach. A complete list of other investigators on this multinational, collaborative effort involved in development, subject management and data collection, supporting industry partners, and people and organizations in Bolivia that made AltitudeOmics possible is available in the first paper in this series (Subudhi *et al*., in review at PLoSOne). The authors are extremely grateful to Mr Jui-lin Fan and Nicolas Bourdillon (University of Geneva, Switzerland), Mr Jonathan Elliot, Dr Steve Laurie, Mr Jim Davis, Ms Julia Kern, Ms Kara Beasley, and Mr Henry Norris (University of Oregon, USA), and Mr Oghenero Evero (University of Colorado, USA) for valuable technical assistance during data collection. Personal thanks go to Professor Alan (Zig) St. Clair Gibson at Northumbria University for making the trip possible for S Goodall.

Grants

AltitudeOmics was funded, in part, by grants from the Department of Defense (W81XWH-11-2-0040 TATRC to RR, and W81XWH-10-2-0114 to ATL); the Cardiopulmonary & Respiratory Physiology Laboratory, University of Oregon; the Altitude Research Center and the Charles S. Houston Endowed Professorship, Department of Emergency Medicine, School of Medicine, University of Colorado Denver; the Faculty of Health & Life Sciences, Northumbria University; the School of Sport and Service Management, University of Brighton; and a National Heart, Lung, and Blood Institute R00 grant (HL-103786 to MA).

Conflict of Interest

Nothing to declare

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Figure Legends

Figure 1. Mean area of motor evoked potentials (MEP) recorded from the vastus lateralis (VL) in response to stimulation over the motor cortex during varying contraction intensities pre- (○) and post-exercise (●) (mean for all conditions). The TMS responses were compared to the area of the maximal M-wave (M_{max}) evoked by peripheral stimulation of the femoral nerve. Data are means ± SE for 7 participants.

Figure 2. Cerebral oxygenation at resting baseline, during the final 30 s of a 3 min warm up (28 W) and during the final 30 s of constant-load exercise (131 W) in normoxia (N; panel a), acute hypoxia (AH; panel b) and chronic hypoxia (CH; panel c). Data are means ± SE for 7 participants. † $P < 0.05$ vs. respective baseline; ‡ $P < 0.05$ vs. respective warm up; * $P < 0.05$ vs. AH; # $P < 0.05$ vs. CH.

Resting baseline in AH denotes the value after 10 min wash in of the hypoxic gas. O_2Hb, oxygenated haemoglobin; HHb, deoxygenated haemoglobin and THb, total haemoglobin.

Figure 3. Arterial oxygen saturation (S_pO_2) (a), cerebral blood flow velocity (MCA_v) (b) and middle cerebral artery O_2 delivery index ($MCA_v \times C_aO_2$) during constant-load exercise (131 W) in normoxia (N), acute hypoxia (AH), and chronic hypoxia (CH). Values are plotted for the duration of the shortest trial (8 min) and extrapolated to the group mean exercise time (10.1 min). Data are means ± SE for 7 participants. † $P < 0.05$ vs. rest; * $P < 0.05$ vs. N; # $P < 0.05$ vs. CH.

Figure 4. Representative MEPs evoked during knee extensor contractions at 50% MVC before exercise in each condition. Traces are shown from a representative participant in each condition; 8 stimuli were delivered from which an average value was obtained. Note the increase in MEP amplitude (corticospinal excitability) after acclimatisation.

Figure 5. Cortical voluntary activation measured before (open bars) and immediately after (<2.5 min; closed bars) constant-load exercise (131 W) in normoxia (N), acute hypoxia (AH), and chronic hypoxia (CH). * $P < 0.05$ pre- vs. post-exercise.

Table 1. Cardiorespiratory and perceptual responses at rest and during the final minute of constant-load cycling (131 W) in normoxia, acute hypoxia and chronic hypoxia.

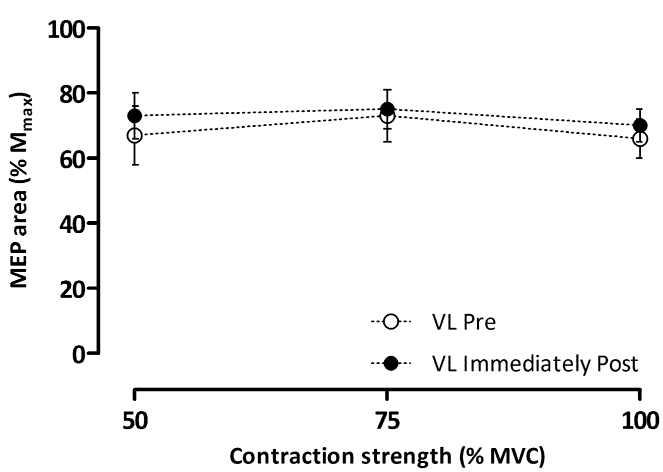
		Normoxia	Acute Hypoxia	Chronic Hypoxia
HR (beats min^{-1})	Rest	81 ± 7†	90 ± 9	104 ± 16
	Final Min	150 ± 16*	173 ± 14	167 ± 16
$\dot{V}_E$ (l min^{-1})	Rest	14.3 ± 2.4	20.0 ± 2.6	24.5 ± 5.4
	Final Min	60.0 ± 9.6*†	108.8 ± 24.7†	128.5 ± 30.0
f_R (breaths min^{-1})	Rest	15.6 ± 3.6	17.5 ± 4.5	13.0 ± 3.4
	Final Min	31.4 ± 4.9*†	51.6 ± 8.7†	54.8. ± 9.9
V_T (l)	Rest	1.07 ± 0.37	1.30 ± 0.34	1.47 ± 0.63
	Final Min	2.00 ± 0.45	2.07 ± 0.43	2.41 ± 0.58
$\dot{V}O_2$ (l min^{-1})	Rest	0.49 ± 0.10	0.45 ± 0.08	0.45 ± 0.12
	Final Min	2.45 ± 0.51	2.34 ± 0.58	2.07 ± 0.50
$\dot{V}CO_2$ (l min^{-1})	Rest	0.44 ± 0.09	0.55 ± 0.09	0.39 ± 0.08
	Final Min	2.32 ± 0.51	2.69 ± 0.62†	1.94 ± 0.50
$\dot{V}_E/\dot{V}O_2$	Rest	30.7 ± 2.7*†	47.4 ± 6.5†	55.9 ± 14.9
	Final Min	25.2 ± 2.4*†	51.2 ± 15.0†	62.9 ± 9.2
$\dot{V}_E/\dot{V}CO_2$	Rest	33.9 ± 2.7†	37.9 ± 6.5†	63.4 ± 6.8
	Final Min	26.2 ± 2.6*†	41.7 ± 6.9†	67.1 ± 9.1
RPE, dyspnoea	Rest	7.0 ± 0.0	7.3 ± 0.5	7.1 ± 0.4
	Final Min	11.4 ± 2.4*†	19.4 ± 0.8	19.1 ± 0.7
RPE, limb	Rest	7.1 ± 0.4	7.1 ± 0.4	7.0 ± 0.0
	Final Min	12.3 ± 3.3*	19.9 ± 0.4	17.6 ± 1.7

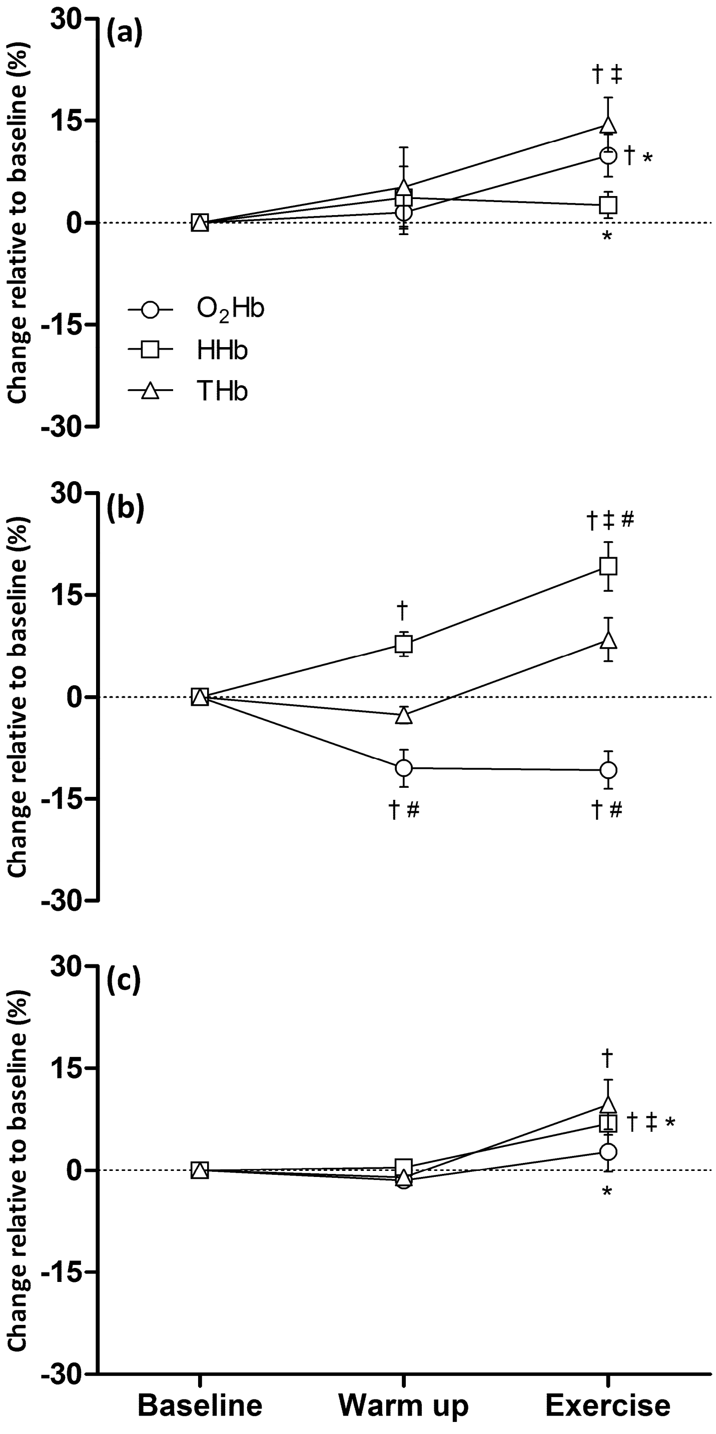
Values are means ± SD for 7 participants. Resting values were measured during the 5th minute of breathing the test gas mixture. HR, heart rate; $\dot{V}_E$, minute ventilation; f_R, respiratory frequency; V_T, tidal volume; $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, carbon dioxide output; RPE, ratings of perceived exertion. * $P < 0.05$ vs. acute hypoxia; † $P < 0.05$ vs. chronic hypoxia.

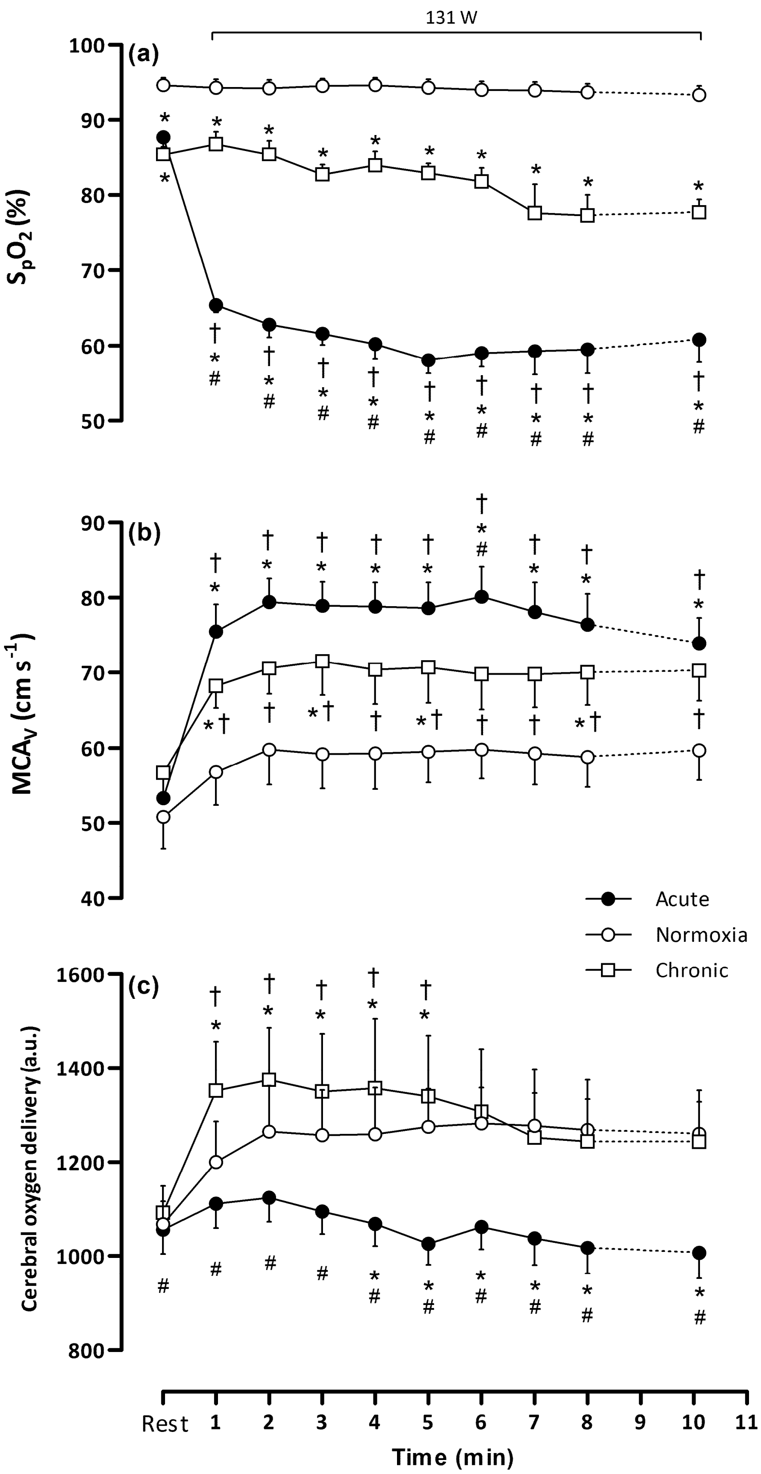
Table 2. Peripheral and central measures of excitability assessed before and after constant-load cycling (131 W) in normoxia, acute hypoxia and chronic hypoxia.

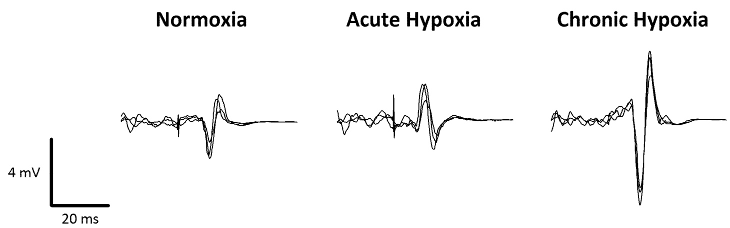
		Normoxia	Acute Hypoxia	Chronic Hypoxia
Rest				
M_{max} amplitude (mV)	Pre	6.9 ± 2.0†	8.6 ± 3.7†	14.9 ± 8.3
	Post	6.7 ± 1.7	9.0 ± 4.1	14.0 ± 8.2
MEP amplitude (mV)	Pre	0.19 ± 0.12†	0.19 ± 0.11†	0.41 ± 0.28
	Post	0.11 ± 0.06	0.11 ± 0.10	0.21 ± 0.18#
MEP/M_{max} (%)	Pre	2.6 ± 1.3	2.7 ± 1.9	4.1 ± 4.2
	Post	1.8 ± 1.2	1.5 ± 1.3	2.6 ± 3.4
Within contraction				
M_{max} amplitude 100% (mV)	Pre	8.9 ± 1.7	9.9 ± 3.2	13.0 ± 6.1
	Post	9.0 ± 1.9	10.0 ± 3.3	11.9 ± 5.4
MEP amplitude 100% (mV)	Pre	3.8 ± 1.5	3.1 ± 1.0†	7.1 ± 4.7
	Post	4.0 ± 2.7	3.2 ± 1.0	6.5 ± 4.4
MEP amplitude 75% (mV)	Pre	3.9 ± 1.5†	2.9 ± 1.4†	7.6 ± 4.9
	Post	4.3 ± 2.6	3.3 ± 1.2†	6.9 ± 3.9
MEP amplitude 50% (mV)	Pre	2.54 ± 0.87†	2.16 ± 0.52†	6.5 ± 4.8
	Post	2.99 ± 2.01†	2.56 ± 0.95†	6.4 ± 4.5
MEP/M_{max} (%) 100% MVC	Pre	35 ± 17	33 ± 14	52 ± 17
	Post	39 ± 20	37 ± 15	52 ± 19
MEP/M_{max} (%) 75% MVC	Pre	40 ± 15	34 ± 19†	58 ± 18
	Post	42 ± 17	38 ± 18†	57 ± 13
MEP/M_{max} (%) 50% MVC	Pre	28 ± 14†	26 ± 10†	50 ± 21
	Post	30 ± 15†	31 ± 17†	54 ± 23
CSP (ms)	Pre	198 ± 58	174 ± 46	186 ± 36
	Post	188 ± 64	171 ± 35	196 ± 51

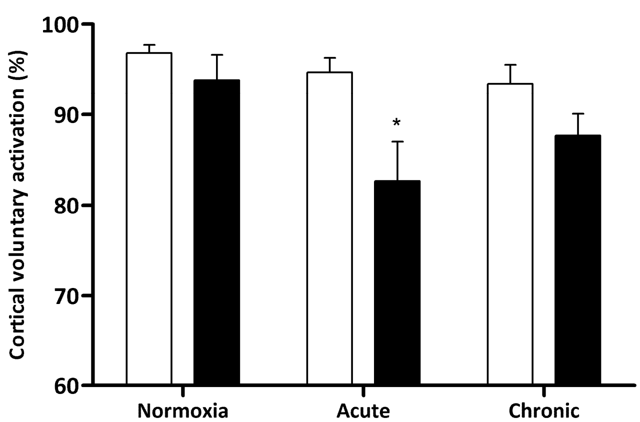
Values are means ± SD for 7 participants. M_{max}, maximal motor response; MEP, motor evoked potential; CSP, cortical silent period. † $P < 0.05$ vs. chronic hypoxia; # $P < 0.05$ vs. Pre.




(a)
(b)
(c)
30
15
0
-15
-30
Change relative to baseline (%)
O2Hb
HHb
THb
† ‡
† *
*
† ‡ #
†
† #
† #
†
† ‡ *
*
Baseline
Warm up
Exercise


131 W
(a)
S_pO_2 (%)
(b)
MCA_V (cm s^{-1})
Acute
Normoxia
Chronic
(c)
Cerebral oxygen delivery (a.u.)
Rest
Time (min)


Normoxia
Acute Hypoxia
Chronic Hypoxia
4 mV
20 ms


Cortical voluntary activation (%)
100
90
80
70
60
*
Normoxia
Acute
Chronic

AltitudeOmics: Effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery

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Running Title: Cerebral oxygen delivery at altitude

Key Words: hypoxia, brain, altitude, cerebral blood flow

Word Count: 4,776

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This is an Accepted Article that has been peer-reviewed and approved for publication in the *Experimental Physiology*, but has yet to undergo copy-editing and proof correction. Please cite this article as an “Accepted Article”; doi: 10.1113/expphysiol.2013.075184.

What is the central question of this study?

Hypoxia associated with ascent to high altitude may threaten cerebral oxygen delivery. We sought to determine if there are regional changes in the distribution of cerebral blood flow that might favor oxygen delivery to areas associated with basic homeostatic functions to promote survival in this extreme environment.

What is the main finding and its importance?

We show evidence of a “brain sparing” effect during acute exposure to high altitude, in which there is a slight increase in relative oxygen delivery to the posterior cerebral circulation. This may serve to support basic regulatory functions associated with the brain stem and hypothalamus.

Abstract

Cerebral hypoxemia associated with rapid ascent to high altitude can be life threatening; yet, with proper acclimatization, cerebral function can be maintained well enough for humans to thrive. METHODS: We investigated adjustments in global and regional cerebral oxygen delivery (DO_2) as 21 healthy volunteers rapidly ascended and acclimatized to 5260m. Ultrasound indices of cerebral blood flow (CBF) in internal carotid and vertebral arteries were measured at sea level (SL), upon arrival at 5260m (ALT1; Pbar = 409mmHg), and after 16 days of acclimatization (ALT16). Cerebral DO_2 was calculated as the product of arterial oxygen content (CaO_2) and flow in each respective artery and summed to estimate global CBF. Vascular resistances were calculated as the quotient of mean arterial pressure and respective flows. RESULTS: Global CBF increased ~70% upon arrival at ALT1 ($P<0.001$) and returned to SL values at ALT16 as a result of changes in cerebral vascular resistance. A reciprocal pattern in CaO_2 maintained global cerebral DO_2 across acclimatization, although DO_2 to the posterior cerebral circulation was increased by ~25% at ALT1 ($P=0.032$). CONCLUSIONS: Cerebral DO_2 is well maintained upon acute exposure and acclimatization to hypoxia, particularly in the posterior and inferior regions of the brain associated with vital homeostatic functions. This tight regulation of cerebral DO_2 was achieved through integrated adjustments in local vascular resistances to alter cerebral perfusion during both acute and chronic exposure to hypoxia.

Introduction

Although the brain represents only about 2% of body weight, it is a highly metabolic tissue that receives ~15% of cardiac output and accounts for ~20% of total body oxygen consumption at rest (Wade & Bishop, 1962). Maintenance of cerebral oxygen delivery (DO_2) is essential for vital cerebral functions associated with homeostasis. In the face of severe hypoxemia, such as experienced during rapid ascent to extreme altitudes (> 8,000 m), reduction in cerebral DO_2 results in loss of consciousness within seconds (Luft *et al.*, 1951; Luft & Noell, 1956) and death within minutes (Bert, 1943). However, with staged acclimatization to progressively higher elevations, cerebral DO_2 can be maintained well enough for humans to reach the summit of Mount Everest (8,848 m) without supplemental oxygen. The mechanisms responsible for this remarkable plasticity in cerebral DO_2 are complex and not completely understood.

Cerebral DO_2 is the product of cerebral blood flow (CBF) and arterial oxygen content (CaO_2). It is well established that CBF rises upon acute exposure to high altitude and returns to near sea-level values with acclimatization (Severinghaus *et al.*, 1966; Huang *et al.*, 1987; Jensen *et al.*, 1990), while CaO_2 decreases in acute hypoxia and returns to sea-level values with acclimatization. These opposing CBF and CaO_2 responses to altitude appear to offset one another and maintain cerebral DO_2 across acclimatization (Severinghaus *et al.*, 1966; Wolff *et al.*, 2002). The pattern of CBF change in response to hypoxia has been attributed to the relative balance of hypoxic vasodilation and hypocapnic vasoconstriction in the brain (Xu & Lamanna, 2006; Brugniaux *et al.*, 2007). During acute, severe hypoxia, vasodilation typically exceeds vasoconstriction, resulting in greater CBF (Mardimae *et al.*, 2012; Willie *et al.*, 2012). With acclimatization, increased ventilatory drive reduces $PaCO_2$ and improves PaO_2, tipping the balance in favor of vasoconstriction and restoring CBF to pre-exposure values. Changes in the $PaO_2/PaCO_2$ ratio have been shown to account for ~40% of the variation in global CBF over acclimatization (Lucas *et al.*, 2011), with other biochemical (e.g. pH, HCO_3^-, nitric oxide) and hematological (e.g. hemoglobin, hematocrit, blood viscosity) factors presumably accounting for the rest of the response (Todd *et al.*, 1994; Tomiyama *et al.*, 1999; Severinghaus, 2001) to maintain global cerebral DO_2.

Recent data demonstrate that acute normobaric hypoxia (i.e. breathing hypoxic gas) affects the regional distribution of CBF within the brain. Data from positron emission tomography (PET) studies show greater perfusion of the brain stem, hypothalamus, thalamus and cerebellum during acute hypoxia, with (Binks *et al.*, 2008) or without (Buck *et al.*, 1998) controlled levels of $PaCO_2$. Regional differences in cerebrovascular reactivity to O_2 and CO_2 have been postulated to control the distribution of CBF. Vascular Doppler studies of the major tributary vessels of the brain suggest that a greater percentage of blood flow may be directed towards the posterior cerebral circulation, including the brain stem, in response to controlled levels of hypoxia and hypocapnia (Sato *et al.*, 2012). From a teleological perspective, this could help preserve vital homeostatic functions at the expense of higher cognitive processing; however, it is unclear whether regional distribution of CBF is similarly affected in hypobaric hypoxia (i.e. high altitude) or if it changes with acclimatization, as not all studies report significant regional differences (Huang *et al.*, 1987; Willie *et al.*, 2012; Willie *et al.*, 2013).

Despite the importance of O_2 supply for cerebral function, longitudinal studies of cerebral DO_2 at high altitude are sparse. In a secondary analysis of data from Severinghaus et al.'s original study of CBF at high altitude, global cerebral DO_2 in four subjects appeared stable and in excess of oxygen demand after 6-12 hours and 3-5 days of exposure to 3,810m (Severinghaus, 2001; Wolff *et al.*, 2002). Using similar methodology (Kety-Schmidt technique), no differences were found in global cerebral DO_2 measured after 5 weeks at 5,260 m and return to sea level (Moller *et al.*, 2002). Unfortunately, these two studies were based on a limited number of observations, which makes it difficult to detect small differences if they existed (type II error), and utilized methodology that can only measure global cerebral DO_2. A more recent MRI study with a larger sample size reported a tendency towards elevation of cerebral DO_2 after subjects returned from 2 days at 3,800 m (Smith *et al.*, 2013), but no measurements of regional cerebral DO_2 were made. Based the limited data to date, it is uncertain if global or regional cerebral DO_2 varies over time at high altitude.

In this study we used vascular Doppler technology in conjunction with arterial blood sampling to allow us to quantify global and regional changes in CBF and cerebral DO_2 in the field as healthy people rapidly ascended and acclimatized to high altitude (5,260 m). We tested the hypothesis that upon acute exposure cerebral DO_2 would be maintained to regions of the brain associated with homeostasis at the expense of other tissues, but that these changes would normalize with acclimatization.

Methods

Subject recruitment and screening

This study was conducted as part of the AltitudeOmics project, for which a detailed description of the protocol is published elsewhere (Subudhi *et al.*, In Review). Briefly, following institutional ethics approval from the Universities of Colorado and Oregon and the US Department of Defense Human Research Protection Office, young, healthy sea-level residents were recruited from the greater Eugene, Oregon area (elevation 128 m). Potential subjects were screened to exclude anyone who was born or had lived at altitudes >1,500 m for more than one year or had traveled to altitudes > 1,000 m in the past 3 months. After obtaining written consent, physical exams and the Army Physical Fitness Test (push ups, sit ups and 3.2 km run) were performed to verify health and fitness status.

Study overview

To evaluate effects of altitude acclimatization on cerebrovascular hemodynamics, subjects were studied on 3 occasions: 1) at sea level (SL, 130 m), 2) upon acute exposure to 5,260 m (ALT1), and 3) after 16 days of acclimatization (ALT16). Specifically, ~4 weeks following SL measurements in Eugene, Oregon, subjects were flown to La Paz, Bolivia. They spent two nights at low altitude (Coroico, Bolivia, 1,525 m) before being driven to the Chacaltaya Research Station at 5,260 m while breathing supplemental oxygen. Acute responses to high altitude were assessed 2 to 4 hours after arrival and cessation of supplemental oxygen (ALT1). Subjects acclimatized to altitudes ranging from 3,800 to 5,260 m over the next 15 days, with a majority of the time (75%) spent at 5,250 m. Measurements were repeated on ALT16.

Instrumentation

Subjects were studied in an upright, seated position with feet on the floor. Arterial blood pressure (ABP) was monitored via a fluid filled pressure transducer (Utah Medical, Salt Lake City, UT, USA) positioned at heart level and attached to a 22-gauge catheter in a radial artery. Blood flow velocity in the left middle cerebral artery was measured by transcranial Doppler ($MCA_{velocity}$: 2MHz probe, Spencer Technologies, Seattle, WA, USA, affixed to a custom-made headset) at depths ranging from 43 to 54 mm. Signal quality was optimized and an M-mode screen shot was recorded to facilitate subsequent probe placements. Arterial saturation was measured on the right side of the forehead by pulse oximetry (Nellcor N-200, Mansfield, MA, USA). Limb lead electrodes were used to measure ECG (ADInstruments BioAmp, Colorado Springs, CO, USA and Sonosite Micromaxx, Bothell, WA, USA). Metabolic variables, including expired ventilation and gas concentrations were assessed via breath-by-breath (Medgraphics PFX, St. Paul, MN, USA and Vacumed UVM, Ventura, CA, USA) and mixing chamber (Oxigraf O_2cap, Mountain View, CA, USA) systems, calibrated with the same 3-L syringe and known concentrations of O_2 and CO_2 prior to each test. Additionally, core temperature was monitored by telemetry pill (CorTemp HQInc., Palmetto, FL, USA) Analog data were sampled and recorded at 200Hz (ADinstruments Powerlab 16/30, Colorado Springs, CO, USA).

Cerebral Blood Flow

After verification of signal quality, resting data were recorded for 10 min while subjects breathed room air. At 6 min, 2 ml of arterial blood was drawn anaerobically for blood gas analysis (described below). During the last 4 min of the resting period, diameter and blood flow velocity in the left internal carotid (ICA: 1.5 cm distal to the carotid bifurcation) and vertebral arteries (VA: between spinous processes of C4 and C5) were recorded over a minimum of 5 cardiac cycles by a registered diagnostic sonographer (SonoSite Micromaxx L25 probe, Bothell, WA, USA). Briefly, vessel diameter from a longitudinal view was identified and measured with digital calipers in synchronization with the ECG tracing to identify systole and diastole. Velocity was measured in the center of the vessel with an insonation angle < 60 degrees and a sample volume maximized for vessel diameter. The peak velocity tracing across cardiac cycles was used for calculation of mean velocity (time averaged peak) and volumetric flow. This procedure was used to verify accurate tracing of the spectral envelop during data collection and results in higher values than the time averaged mean method (Schoning *et al.*, 1994). All data were downloaded in DICOM format for verification of measurements offline (Sante DICOM Editor, Athens, Greece).

Regional blood flow (ml/min) in the ICA and VA (ICA_{flow} and VA_{flow}) was determined using standard, validated ultrasound techniques (Hoskins, 2008), where:

$$X_{Flow} = \pi^*(\text{diameter in cm}/2)^2 * \text{time averaged peak velocity in cm/s} * 60\text{ s}.$$

Average coefficients of variation determined from three repeated measurements of ICA and VA flow measurements in 7 subjects at SL were 4.0 ± 2.6% and 4.0 ± 2.1%, respectively. Global CBF (gCBF) was estimated assuming symmetrical bilateral flow in the major tributary arteries of the brain (Ogoh *et al.*, 2013; Willie *et al.*, 2013) as:

$$gCBF = (ICA_{flow} + VA_{flow})^*2.$$

Regional and global measurements of CBF were also expressed relative to estimates of cardiac output (%Q) derived from simultaneous intra-arterial blood pressure tracings (Bogert *et al.*, 2010). Cerebral vascular resistance index (CVRi) was calculated as:

$$CVRi = \text{mean ABP}/X_{flow}$$

Cerebral Oxygen Delivery

Arterial blood was immediately analyzed for PaO_2, $PaCO_2$ (Siemens RAPIDLab 248, Erlangen, Germany), [Hb], SaO_2 (Radiometer OSM3, Copenhagen, Denmark) and Hct (M24 Centrifuge, LW Scientific, Lawrenceville, GA, USA). Blood gases were temperature corrected (Kelman & Nunn, 1966; Severinghaus, 1966). CaO_2 (vol%) was calculated as:

$$CaO_2 = 1.39 \times [Hb] + PaO_2 * 0.003$$

Regional and global cerebral DO_2 were calculated as the products of CaO_2 and ICA_{flow}, VA_{flow}, and gCBF.

Data Analysis

After verification of normality, mixed repeated measures ANOVA's were used to analyze the interaction of time by sex for each variable of interest ($\alpha = 0.05$). Subsequent estimation-maximization and multiple-imputation (5 trials) analyses verified negligible effects of missing values (SPSS 20, IBM, Chicago, IL, USA). Paired t-tests (without imputation of missing values) were used for *post hoc* comparisons with the Holm procedure to control for Type I error. A priori power calculations ($\alpha = 0.05$, $\beta = 0.20$) were integrated into the study design to limit Type II error. Pearson product moment correlations were used to describe shared variance between variables. Data are presented as mean ± SD.

Based on the hypothesis that increased CBF may play a role in the pathogenesis of acute mountain sickness [AMS (Jensen *et al.*, 1990; Baumgartner *et al.*, 1994; Baumgartner *et al.*, 1999)], a secondary analysis was performed to evaluate potential relationships (Spearman correlations) between changes in CBF and DO_2 with the severity of Lake Louise Questionnaire (LLQ) symptoms scores reported in these subjects on ALT1 (Subudhi et al. – in review). Paired t-tests were used to evaluate differences in CBF and DO_2 between those with severe AMS (LLQ ≥ 6 including headache) and those remaining healthy.

Results

Subject Characteristics

Detailed baseline characteristics of the 21 (12 males and 9 females; 21 ± 1 years old) subjects participating in AltitudeOmics are presented elsewhere (Subudhi *et al.*, In Review). Males exhibited greater [Hb], CaO_2 and DO_2 than females over the course of the study (all $P < 0.05$), but since no interactions in CBF or DO_2 were detected across acclimatization, combined data are presented below.

Cerebral Blood Flow and Oxygen Delivery

Acute exposure to 5,260 m (Pbar = 408 ± 1 mmHg) decreased PaO_2, SaO_2 and CaO_2 by 66.1 ± 5.4 mmHg, 22 ± 6%, and 4.1 ± 1.2 ml/dl, respectively (all $P<0.001$; Table 1). This severe degree of hypoxia increased heart rate 14 ± 11 bpm ($P < 0.001$) without affecting mean ABP (P=0.380). CBF increased 74 ± 81% in the ICA (P = 0.018), 59 ± 54% in the VA (P = 0.001), and 69 ± 57% globally (P = 0.003). Respective CVRi values fell (all P< 0.001; Table 2), allowing a larger percentage of cardiac output to perfuse the brain (P = 0.010). Increased ICA_{flow} was characterized by increased ICA velocity (P = 0.004) without a change in diameter (P =0.068), while increased VA_{flow} was explained by an increase in VA diameter (P = 0.005) without a change in velocity (P=0.120). $MCA_{velocity}$ was unchanged (P = 0.953). Increased gCBF offset the decrease in CaO_2 to maintain global cerebral DO_2 (Figure 1), although a small increase in VA DO_2 was observed (P=0.039, Figure 2). Observed changes in measures of regional and global CBF and DO_2 were not correlated with LLQ scores of AMS (r = -0.07 to -0.23, P = 0.38 to 0.78), nor were they different between those reporting severe AMS and those remaining healthy (P = 0.57 to 0.97).

Following acclimatization, a 32 ± 36% rise in ventilation was accompanied by a 5.5 ± 2.7 mmHg decrease in $PaCO_2$ and 9.2 ± 4.1 mmHg increase in PaO_2 (ALT1 vs. ALT16; all P < 0.001). SaO_2 and [Hb] rose 6 ± 5% and 1.8 ± 0.9 g/dL, respectively, improving CaO_2 by 3.1 ± 1.2 ml/dl (all P < 0.001; Table 1). ABP was unaffected by acclimatization (ALT1 vs. ALT16; P=0.211). ICA_{flow}, VA_{flow} and gCBF returned to SL values (SL vs. ALT16; P = 0.810, 0.977, 0.620, respectively; Table 2). Respective CVRi values increased as both ICA and VA diameters decreased from ALT1 to ALT16 (all P < 0.020) and restored the relative distribution of cardiac output back to SL values (SL vs. ALT16; P = 0.121). Cerebral DO_2 fell from ALT1 to ALT16 (ICA DO_2 P = 0.028, VA DO_2 P = 0.020, global DO_2 P = 0.011) as the reductions in CBF outweighed the increase in CaO_2 (Figure 1); however, neither global nor regional cerebral DO_2 values fell below that measured at SL (all P > 0.420; Figures 1 & 2).

Discussion

This is the first study to assess regional cerebral oxygen delivery in the field over a period of acclimatization to high altitude. Our findings confirm that global cerebral DO_2 was preserved across acclimatization through a changing balance between CBF and CaO_2, but there was slight increase in relative DO_2 to the posterior cerebral circulation during acute exposure. Although changes in CBF and DO_2 were not associated with the incidence or severity of AMS, regional regulation of CBF may serve to support vital homeostatic cerebral functions in hypoxia.

Preservation of Cerebral Oxygen Delivery

The increase in CBF upon arrival at high altitude and decrease back to sea level values with acclimatization was opposed by changes in CaO_2 (Figure 2). These responses preserved cerebral DO_2 close to sea level values and affirm that components of CaO_2 (PaO_2, SaO_2, [Hb]) outweigh the influence $PaCO_2$ in regulating CBF in severe hypoxia. Increased CBF upon arrival at high altitude resulted from reduced cerebral vascular resistance rather than increased blood pressure (Tables 1&2). Although reduction in vascular resistance is commonly attributed to dilation of pial and parenchymal arterioles in the brain (Fog, 1938), we observed increased diameter of larger tributary arteries, supporting a global vascular response to this degree of hypoxia (Heistad *et al.*, 1978; Faraci & Heistad, 1990; Willie *et al.*, 2012). Mechanisms governing hypoxic vasodilation are complex, involving local (e.g. astrocyte regulation, nitric oxide) and diffuse (e.g. central chemoreception, autonomic nervous system) mechanisms, but all stem from a reduction in PaO_2 (Severinghaus, 2001; Xu & Lamanna, 2006). When PaO_2 is above 60 mmHg, little vasodilation is evident (Mardimae *et al.*, 2012; Willie *et al.*, 2012). Below this threshold, the degree of vasodilation increases exponentially and outweighs the degree of hypocapnic vasoconstriction (Mardimae *et al.*, 2012; Willie *et al.*, 2012) - presumably to provide greater blood flow in a time of need. While the correlation between changes in gCBF and CaO_2 was not significant, the change in CaO_2 from SL to ALT1 was similar among all subjects and may not have afforded an appropriate range of values to detect the relation that has previously been shown with progressive hemodilution (Korosue & Heros, 1992). Qualitatively, the ~70% increase in gCBF was within the expected range during acute hypocapnic hypoxia (Severinghaus, 1966; Jensen *et al.*, 1990; Severinghaus, 2001; Brugniaux *et al.*, 2007) and proportional to the ~60% reduction in PaO_2 that was responsible for the reduction in CaO_2. This reciprocal relationship, whether evolved or serendipitous, is advantageous for survival in these extreme conditions as it mitigates negative consequences of cerebral hypoxemia.

Although increased CBF has been suggested to play a role in the pathogenesis of AMS (Baumgartner *et al.*, 1994), our results were more similar to those refuting the hypothesis (Jensen *et al.*, 1990; Baumgartner *et al.*, 1999). Regional and global CBF and DO_2 measurements were not correlated with AMS symptoms scores and did not differentiate between those with severe AMS and those who remained healthy after rapid ascent to high altitude. Nonetheless, our data should be interpreted with caution since it is possible that increased CBF contributes to the development of AMS when other, yet to be described, factors are present.

Increased PO_2 and decreased PCO_2 after 16 days at high altitude are hallmarks of ventilatory acclimatization that are addressed elsewhere (Fan et al. in review). As a result, PaO_2-mediated dilation was reduced and $PaCO_2$-mediated vasoconstriction was increased, thereby lowering CBF. Assuming a cerebral O_2 reactivity of 3% CBF /

% SaO_2 and a CO_2 reactivity of 4% CBF / mmHg CO_2 from a previous duplex ultrasound study (Willie *et al.*, 2012), we could account for the entire decrease in gCBF across acclimatization. Specifically, the 5% increase in SaO_2 could be expected to reduce CBF by ~15% and the 5.5 mmHg decrease in $PaCO_2$ could be expected to reduce CBF by ~22%, thus accounting for the 36% decrease in gCBF we observed from ALT1 to ALT16 (Table 2). We acknowledge that increased cerebrovascular CO_2 reactivity with acclimatization in our subjects (Fan et al. in review) may account for an even greater proportion of the net effect on CBF at ALT16. Also, the relative influence of other hematological factors, such as increased hematocrit and blood viscosity (Sorensen *et al.*, 1974; Todd *et al.*, 1994; Tomiyama *et al.*, 1999) from erythropoiesis and plasma volume contraction, may have contributed to the reduction of CBF across acclimatization (data to be presented elsewhere). Yet our data suggest that the inherent vascular reactivities to O_2 and CO_2 are sufficient to maintain tight control over cerebral DO_2 in hypoxia. Consistent delivery of oxygen may help offset the decreased PO_2 gradient (plasma to mitochondria) and support the cerebral metabolic demand for oxygen at this altitude (Severinghaus *et al.*, 1966; Moller *et al.*, 2002) to preserve cerebral function. Together, our data demonstrate that integrated mechanisms controlling cerebral blood flow are well suited to preserve global cerebral oxygen delivery at 5,260 m.

Regional Cerebral Oxygen Delivery

We observed a small increase in DO_2 through the posterior cerebral circulation upon arrival at high altitude (Table 2) that dissipated with acclimatization. The acute increase in DO_2 was characterized by an increase in VA diameter and supports recent findings of greater VA (vs. ICA) vasoreactivity during acute hypoxia (Willie *et al.*, 2012; Ogoh *et al.*, 2013). Of note, Ogoh et al. (Ogoh *et al.*, 2013), showed that acute hypoxia (~15 min) increased VA, but not ICA, blood flow. Since the areas perfused by the VA include the brainstem, and posterior aspects of the thalamus and hypothalamus, increased blood flow and DO_2 to these regions during acute hypoxia (Buck *et al.*, 1998; Binks *et al.*, 2008) may be seen as necessary to maintain vital homeostatic functions (Sheldon *et al.*, 1979; Bilger & Nehlig, 1993). Since increased cardiorespiratory drive with acclimatization was not associated with a continued elevation of VA DO_2, we speculate that the increased VA DO_2 during acute hypoxia was protective, to defend against a potential threat in oxygen supply, rather than to merely support neuronal metabolic activity associated with heightened autonomic activity (i.e. neurovascular coupling). Although such hypothetical explanations for regional differences in the regulation of CBF and DO_2 are intriguing, our results must be interpreted with caution since measured differences were small and are not consistently reported in the literature (Huang *et al.*, 1987; Willie *et al.*, 2013). Future studies with more focal measurements of DO_2 (e.g. PET and MRI) and neuronal activity in key regulatory regions of the brain, as well as measurements of neurovascular coupling (as an index of neuronal plasticity) during acute and prolonged hypoxia are needed to yield further insight into this question.

Brain Sparing

Reduced cerebral vascular resistance associated with vasodilation upon arrival at altitude can explain the proportional increase in CBF and greater allocation of cardiac output. This effect could be magnified if there is net constriction in other vascular beds at rest. Previous studies have shown that superior mesenteric and renal artery blood flow decrease in acute hypoxia and could allow for greater perfusion of the brain (Greene & Roach, 2004). With acclimatization, cerebral vascular resistance and blood flow returned to sea-level values. These results are similar to fetal 'brain sparing' effects (Campbell *et al.*, 1967; Peeters *et al.*, 1979; Sheldon *et al.*, 1979) that are presumed to preserve vital homeostasis during hypoxia in utero (Pearce, 2006; Salihagic-Kadic *et al.*, 2006). Similar effects have also been shown in newborn dogs (Cavazzuti & Duffy, 1982), piglets (Goplerud *et al.*, 1989), and premature infants (Daven *et al.*, 1983). The largest response to hypoxia tends to occur in the brainstem during the early postnatal period and decreases with age (Bilger & Nehlig, 1993). We are the first to demonstrate that such a 'brain sparing' reaction exists in healthy human adults exposed to acute hypoxia and recedes with acclimatization. Preferential distribution of cardiac output to the brain upon acute altitude exposure may represent a conserved mechanism that protects against hypoxic brain damage in mammals, particularly in regions associated with basic cardiovascular and respiratory control during periods of acute hypoxia. Measurements of regional cerebral metabolism are needed to determine if 'brain sparing' effectively matches DO_2, or if the increase in CBF represents a protective form of overcompensation.

Limitations

Our rapid ascent profile in combination with supplemental oxygen during transport from low to high altitude was designed to induce an abrupt change in PaO2, similar to that which can be achieved in laboratory studies with hypoxic gas or hypobaric chambers. As such, our results must be interpreted in this context and thus may be expected to be different from other field studies that have followed more traditional progressive ascents (Huang *et al.*, 1987; Jensen *et al.*, 1990; Baumgartner *et al.*, 1994; Willie *et al.*, 2013).

We used duplex sonography primarily because it is a non-invasive technique that can be utilized in field settings. This technique yields volumetric measurements, in terms of ml/min, which, based on first principles, can be multiplied by CaO_2 to yield DO_2. Our low CVs were in line with a previous study showing similarity between duplex sonography and both PET and xenon inhalation methods of measuring gCBF (Schoning & Scheel, 1996). Nevertheless, we acknowledge that all these techniques are limited by the lack of an absolute standard for validating CBF. Our gCBF measurements were based on unilateral, left-sided measurements of the ICA and VA – the main arteries perfusing the brain. While left VA flow has been reported to be ~20% higher than the right (Schoning *et al.*, 1994), this was not expected to have an effect on global measurements since ICA flow represents the majority of gCBF (Schoning & Scheel, 1996). Yet, unilateral VA measurements may have influenced our finding of increased VA DO_2. Future studies are needed to determine if 'brain sparing' effects are attenuated when independent measurements of left and right VA flow are summed.

Since the ICA feeds the MCA, we expected that changes in ICA flow would be reflected in $MCA_{velocity}$. This was not the case: ICA flow increased ~70% while

$MCA_{velocity}$ was unchanged throughout the study. A similar discrepancy between ICA flow and $MCA_{velocity}$ has been previously described by Willie et al. (Willie *et al.*, 2012) and argued to support dilation of the MCA in hypoxia (Wilson *et al.*, 2011). We calculated that a 12% increase in MCA diameter could explain the measured discrepancy between ICA_{flow} and $MCA_{velocity}$. This exact degree of vasodilation has recently been demonstrated at high altitude with a color-coded ultrasound technique (Willie *et al.*, 2013), yet because additional studies are needed to clarify artery-specific responses to hypoxia and validate MCA-diameter measurement techniques, we chose to refrain from further interpretation of $MCA_{velocity}$.

Summary & Implications

Overall, our findings highlight the integrative nature of responses that preserve oxygen delivery to the brain at high altitude. Regional cerebral vasoreactivity to O_2 and CO_2 may favor oxygen delivery to posterior and inferior regions of the brain during acute hypoxia to sustain vital cerebral functions associated with homeostasis. Whether these mechanisms evolved to promote survival in conditions provoking cerebral hypoxia is not clear at present, but further research in this area may yield important insights into human tolerance and adaptation to chronic states of hypoxemia.

Competing Interests

The authors have no conflicts or competing interests to disclose.

Funding

The overall AltitudeOmics study was funded, in part, by grants from the U.S. Department of Defense (W81XWH-11-2-0040 TATRC to RR, and W81XWH-10-2-0114 to ATL); the Cardiopulmonary & Respiratory Physiology Laboratory, University of Oregon; the Altitude Research Center and the Charles S. Houston Endowed Professorship, Department of Emergency Medicine, School of Medicine, University of Colorado Denver Anschutz Medical Campus.

Acknowledgements

This paper is part of a series titled "AltitudeOmics" that together represent a group of studies that explored the basic mechanisms controlling human acclimatization to hypoxia and its subsequent retention. Many people and organizations invested enormous amounts of time and resources to make AltitudeOmics a success. Foremost, the study was made possible by the tireless support, generosity and tenacity of our research subjects. AltitudeOmics principal investigators were Colleen G. Julian, Andrew T. Lovering, Andrew W. Subudhi and Robert C. Roach. A complete list of other investigators on this multinational, collaborative effort involved in development, subject management and data collection, supporting industry partners, and people and organizations in Bolivia that made AltitudeOmics possible is available elsewhere(Subudhi *et al.*, In Review).

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Table 1. Cardiopulmonary and hematological values [mean ±SD (n)]

Variable		SL	ALT1	ALT16
VE	l/min	12.05 ± 2.50 (21)	11.93 ± 2.92 (17)	14.88 ± 2.65 (21)*#
PaO2	mmHg	102.2 ± 5.5 (21)	36.1 ± 2.8 (18)*	45.3 ± 3.2 (20)*#
PaCO2	mmHg	38.1 ± 4.4 (21)	26.5 ± 3.1 (18)*	20.9 ± 2.5 (20)*#
SaO2	%	98 ± 1 (21)	76 ± 6 (18)*	82 ± 3 (20)*#
[Hb]	g/dl	13.9 ± 1.4 (21)	14.2 ± 1.5 (18)*	16.0 ± 2.0 (20)*#
CaO2	ml/dl	19.4 ± 1.9 (21)	15.2 ± 2.1 (18)*	18.4 ± 2.4 (20)*#
HR	bpm	76 ± 12(21)	90 ± 16 (16)*	96 ± 13 (20)*
SV	ml	91 ± 27 (21)	85 ± 20 (16)	83 ± 21 (20)
Mean ABP	mmHg	79 ± 8 (21)	76 ± 13 (16)	80 ± 10 (20)

* Different from SL
# Different from ALT1

Table 2. Cerebrovascular values [mean± SD (n)]

Variable		SL	ALT1	ALT16
ICA Dia	cm	0.51 ± 0.08 (21)	0.54 ± 0.07 (16)	0.50 ± 0.07 (20)#
ICA Vel	cm/s	29.8 ± 8.2 (21)	38.9 ± 8.1 (16)*	32.1 ± 5.4 (20)#
ICA Flow	ml/min	384 ± 197 (21)	556 ± 203 (16)*	379 ± 97(20)#
ICA CVRi	mmHg/ml/min	0.25 ± 0.12 (21)	0.16 ± 0.09 (16)*	0.23 ± 0.07 (19)#
VA Dia	cm	0.36 ± 0.06 (20)	0.41 ± 0.06 (16)*	0.36 ± 0.06 (19)#
VA Vel	cm/s	21.4 ± 4.4 (20)	24.4 ± 6.4 (16)	19.3 ± 7.1 (19)#
VA Flow	ml/min	133 ± 47 (20)	206 ± 98 (16)*	122 ± 55 (19)#
VA CVRi	mmHg/ml/min	0.66 ± 0.24 (20)	0.46 ± 0.28 (16)*	0.84 ± 0.58 (19)#
gCBF	ml/min	1057 ± 413 (20)	1524 ± 456 (16)*	981 ± 223 (19)#
gCBF CVRi	mmHg/ml/min	0.09 ± 0.03 (20)	0.05 ± 0.02 (16)*	0.08 ± 0.02 (19)#
DO2 ICA	ml/min	75 ± 37 (21)	84 ± 32 (16)	68 ± 19 (19)#
DO2 VA	ml/min	26 ± 10 (20)	31 ± 16 (16)*	22 ± 11 (19)#
DO2 gCBF	ml/min	206 ± 79 (20)	230 ± 74 (16)	181 ± 51 (19)#
MCAv	cm/s	59.5 ± 10.3 (21)	61.1 ± 13.3 (17)	57.7 ± 7.1 (21)
MCA CVRi	mmHg/cm/s	1.36 ± 0.25 (21)	1.28 ± 0.32 (17)	1.41 ± 0.24 (20)
ICA %Q	%	5.4 ± 2.7 (21)	7.6 ± 2.7 (15)*	4.8 ± 1.4 (18)#
VA %Q	%	1.9 ± 0.8 (20)	2.6 ± 1.1 (15)*	1.5 ± 0.7 (18)#
gCBF %Q	%	15.0 ± 5.8 (20)	20.4 ± 6.2 (15)*	12.6 ± 3.4 (18)#

* Different from SL
# Different from ALT1

Figure 1. Reciprocal changes in global cerebral blood flow (gCBF) and arterial oxygen content (CaO2) maintained global cerebral oxygen delivery (DO2) across the study. * Different from sea level (SL). # Different from arrival at altitude (ALT1).

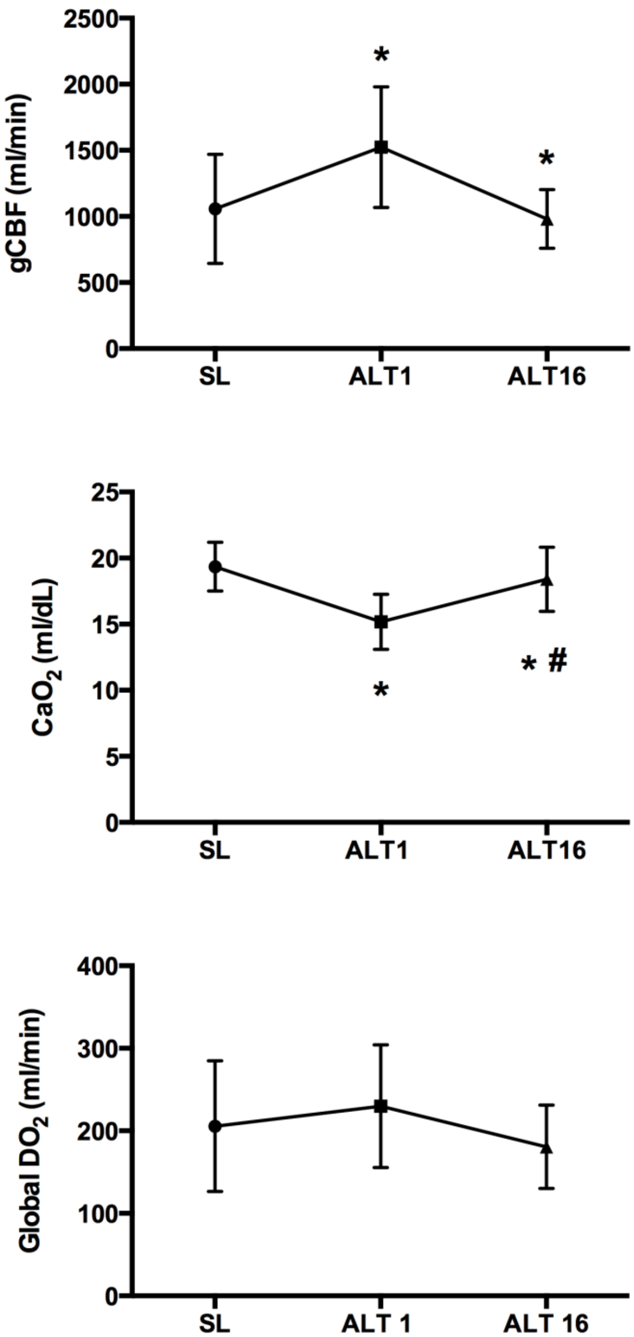
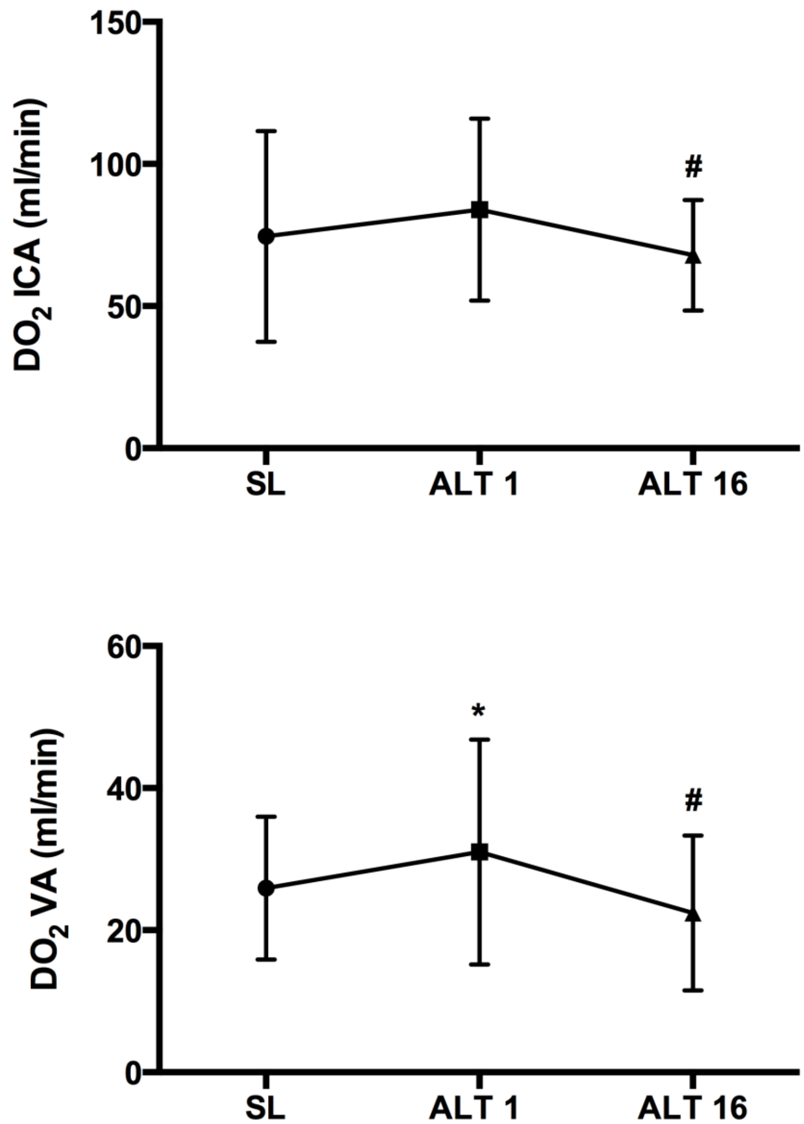


Figure 2. Regional oxygen delivery (DO_2) increases in the vertebral artery (VA), but not internal carotid artery (ICA) at ALT1. Regional DO_2 is reduced with acclimatization, but not below sea level (SL) values. * Different from SL. # Different from arrival at altitude (ALT1).



Articles in PresS. J Appl Physiol (December 19, 2013). doi:10.1152/japplphysiol.00704.2013

AltitudeOmics: Enhanced cerebrovascular reactivity and ventilatory response to CO_2 with high altitude acclimatisation and re-exposure

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Running head: Cerebral function at altitude

Key words: cerebral blood flow, cerebral CO_2 reactivity, rebreathing, altitude acclimatisation

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Word count: 5,010

Abstract

The present study is the first to examine the effect of high altitude acclimatisation and re-exposure on the responses of cerebral blood flow and ventilation to CO_2. We also compared the steady-state estimates of these parameters during acclimatisation with the modified rebreathing method. We assessed changes in steady state responses of middle cerebral artery velocity (MCAv), cerebrovascular conductance index (CVCi) and ventilation ($\dot{V}E$) to varied levels of CO_2 in 21 lowlanders (9 females; 21 ± 1 years), at sea-level (SL), during initial exposure to 5,260m (ALT1), after 16 days of acclimatisation (ALT16) and upon re-exposure to altitude following either 7 (POST7) or 21 days (POST21) at low altitude (1,525m). In the non-acclimatised state (ALT1), MCAv and $\dot{V}E$ responses to CO_2 were elevated compared to SL (by 79±75% and 14.8±12.3 L/min, respectively, P=0.004 & P=0.011). Acclimatisation at ALT16 further elevated both MCAv and $\dot{V}E$ responses to CO_2 compared to ALT1 (by 89±70% and 48.3±32.0 L/min, respectively, P<0.001). The acclimatisation gained for $\dot{V}E$ responses to CO_2 at ALT16 was retained by 38% upon re-exposure to altitude at POST7 (P=0.004 vs. ALT1), while no retention was observed for the MCAv responses (P>0.05). We found good agreement between steady-state and modified rebreathing estimates of MCAv and $\dot{V}E$ responses to CO_2 across all three time points (P<0.001, pooled data). Regardless of the method of assessment, altitude acclimatisation elevates both the cerebrovascular and ventilatory responsiveness to CO_2. Our data further demonstrates that this enhanced ventilatory CO_2 response is partly retained after 7 days at low altitude.

Introduction

The ability to maintain adequate oxygen transport to the brain by cerebral blood flow (CBF) in hypoxic environments is vital. The CBF responsiveness to CO_2, termed cerebrovascular CO_2 reactivity, provides a useful, non-invasive index of cerebrovascular function (3, 19). To date, only a handful of studies have investigated the effect of acclimatisation to high altitude on cerebrovascular CO_2 reactivity (1, 16, 17, 24, 30, 49). The interpretation of findings from these studies is difficult due to the timing of measurements at high altitude (1, 16, 17, 24, 25), the confounding effects of previous high-altitude exposure (1), artificial normobaric hypoxia (28, 46), and the method used to assess reactivity (24, 30, 49). Data from Fan et al., (16, 17), obtained on subjects at different stages of altitude acclimatisation, suggest that cerebrovascular CO_2 reactivity is elevated with prolonged exposure to high altitude when using a modified rebreathing technique. In contrast, Lucas et al., (30) reported a reduced cerebrovascular CO_2 reactivity in the same subjects that at the end of a 14 day stay at 5,050 m, when assessed with a steady-state technique (poikilocapnic hypoxia). More recently, Rupp et al., (49) reported a reduced cerebrovascular CO_2 reactivity during steady-state hypoxic hypercapnia following 5 days at 4,350 m. Thus, the effect of altitude acclimatisation on cerebrovascular CO_2 reactivity remains unclear.

In addition, it is unknown if and for how long changes in cerebrovascular CO_2 reactivity from acclimatisation persist after descent. Repetitive seven-month exposures to high altitude were reported to improve arterial O_2 saturation (SaO_2), lower resting heart rate (HR) and decrease susceptibility to acute mountain sickness (AMS) upon subsequent re-exposures (59). Remarkably, these prior-exposure adaptations persisted despite a five-month deacclimatisation period. The specific effect of high altitude re-exposure on cerebrovascular and ventilatory responsiveness to CO_2 has yet to be examined.

Changes in cerebrovascular CO_2 reactivity with high-altitude acclimatisation depend on the method of assessment. At sea level, the steady-state method results in higher cerebrovascular CO_2

reactivity (40-42) and lower ventilatory CO_2 sensitivity (6, 18, 23, 55) compared to the modified rebreathing test. These differences have been attributed to the presence of a PCO_2 gradient (between alveolar, arterial, and cerebrospinal fluid compartments) during the steady-state method, which is supposedly abolished or minimised during rebreathing (6). Meanwhile, elevated basal $\dot{V}E$ and subsequent underestimation of the ventilatory CO_2 sensitivity has been proposed as one possible explanation for lower steady-state estimates (34). No studies have directly compared the steady-state and modified rebreathing test estimates of cerebrovascular and ventilatory CO_2 responsiveness following ascent or acclimatisation to high altitude.

The purpose of the present study was therefore two-fold: first, we wished to assess the effect of altitude exposure on cerebrovascular and ventilatory responsiveness to CO_2 in acute conditions, after acclimatisation and upon re-exposure to high altitude after a period spent at low altitude; second, we wished to compare the steady-state and modified rebreathing methods for assessing the ventilatory and cerebrovascular responsiveness to CO_2 at high altitude.

Methods

Subject recruitment and screening

This study was conducted as part of the AltitudeOmics project. Following institutional ethics approval, young (19-23 years old), healthy, sea level residents were recruited from the greater Eugene, Oregon area (elevation 130 m). Potential subjects were screened to exclude anyone who was born or had lived at altitudes >1500 m for more than one year or had travelled to altitudes >1000 m in the past 3 months. A detailed description of subject recruitment procedures, including inclusion and exclusion criteria have been presented elsewhere (54).

Ethical approval

The study was performed according to the *Declaration of Helsinki* and was approved by the Institutional Review Boards of the Universities of Colorado and Oregon and by the Human Research Protection Office of the U.S. Department of Defense. All participants were informed regarding the procedures of this study, and written informed consent was given prior to participation.

Experimental Design

After familiarisation with the experimental procedures outlined below (visit one), the subjects underwent experimental trials near sea level (SL: 130 m, barometric pressure: 749 mmHg) and three times at high altitude (5,260 m, Mt Chacaltaya, Bolivia; barometric pressure 406 mmHg); on the 1st and 16th days at high altitude (ALT1 and ALT16) and again after either 7 (POST7; n=14) or 21 (POST21; n=7) days at low altitude (1,525 m, barometric pressure: 639 mmHg). An overview of the entire experimental design and protocol has been described in detail elsewhere (54).

Experimental protocol

For each subject, all ALT measurements were carried out around the same time of day to minimise any confounding effect of circadian rhythm. Measurements were taken upon arrival at ALT1 to minimise the influence of AMS. Likewise, no symptoms of AMS were observed at ALT16 or POST7.

For this study, following 10-15 min of quiet rest in a seated position, each experimental testing session comprised of: a) instrumentation; b) 10 min room air baseline; and c) cerebrovascular CO_2 reactivity tests. The cerebrovascular CO_2 reactivity tests consisted of: i) 10 min with end-tidal PCO_2 ($PETCO_2$) clamped at 40 mmHg; ii) 3 min voluntary hyperventilation to lower $PETCO_2$ to ~20 mmHg; iii) the modified rebreathing test (details below); and iv) 3 min with

$PETCO_2$ clamped at 50 mmHg. The entire cerebrovascular CO_2 reactivity protocol was carried out in background of hyperoxia (end-tidal PO_2 [$PETO_2$] > 250 mmHg).

Experimental setup

Throughout the protocol, the subjects sat upright and breathed through a mouthpiece attached to a two-way non-rebreathing valve (Hans-Rudolph 2700, Hans-Rudolph Inc., Shawnee, KS, USA). The breathing circuit allowed switching from room air to either an end-tidal clamping system or a rebreathing system. The end-tidal clamping setup used in the present study is a modified version of the system previously described by Olin et al., (39). The setup allowed stabilising $PETCO_2$ at 40 and 50 mmHg. Throughout the end-tidal PCO_2 clamping, we maintained $PETO_2$ at >250 mmHg by titrating 50% or 100% O_2 into the inspiratory reservoir at SL and ALT, respectively.

Modified rebreathing method

The modified rebreathing method is a well-established method for assessing both ventilatory and cerebrovascular CO_2 reactivities (14, 16, 34, 41). By using hyperoxia ($PETO_2$ > 250 mmHg) the test minimises peripheral chemoreceptors' output (11, 21) and the ventilatory response to the modified rebreathing method can thus 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., (16, 17). The rebreathing bag was filled with gas to achieve inspired PCO_2 and PO_2 of 0 mmHg and 300 mmHg, respectively, at each altitude. Subjects were instructed to hyperventilate for 3 min (part ii) to lower and then maintain $PETCO_2$ at 20 mmHg at both sea level and 5,260 m (in background $PETO_2$ > 250 mmHg). Subjects were then switched to the rebreathing bag, and following two initial deep breaths to mix the gas from the bag with that in the respiratory system, they were instructed to breathe *ad libitum* (part iii). The rebreathing tests were terminated when $PETCO_2$ reached 50 mmHg, $PETO_2$ dropped below 200 mmHg or the subject reached the end of his/her hypercapnic tolerance.

Measurements

Cerebrovascular variables: Middle cerebral artery velocity (MCAv, an index of cerebral blood flow) was measured in the left middle cerebral artery using a 2-MHz pulsed Doppler ultrasound system (ST3, Spencer technology, Seattle, WA, USA). The Doppler ultrasound probe was positioned over the left temporal window and held in place with an adjustable plastic headband (Marc 600 Headframe, Spencer technology, Seattle, WA, USA). The signal was acquired at depths ranging from 43 to 54 mm. Signal quality was optimised and an M-mode screen shot was recorded to facilitate subsequent probe placements. Peripheral saturation was measured on the right side of the forehead by pulse oximetry (N-200, Nellcor Inc., Hayward, CA, USA).

Cardiovascular variables: Beat-to-beat mean arterial blood pressure (MAP) was measured from an arterial catheter inserted in a radial artery, and connected to a calibrated, fluid-filled, disposable pressure transducer positioned at the level of the heart (DELTRAN II, Utah Medical, Salt Lake City, UT, USA). Heart rate (HR) was determined with a three-lead ECG (ADInstruments BioAmp & Micromaxx, SonoSite Inc., Bothell, WA, USA). Cerebrovascular conductance index (CVCi) was calculated using the equation CVCi = MCAv/MAP and normalised to values obtained at a $PETCO_2$ of 20 mmHg and expressed as percentage change.

Respiratory variables: $\dot{V}E$ was measured using a pneumotachograph (Universal Ventilation Meter, Vacu•Med, Ventura, CA, USA; Ultima™ series, Medgraphics CPX, Minneapolis, MN, USA) and expressed in units adjusted to BTPS. $PETO_2$ and $PETCO_2$ were measured using fast responding gas analysers (O_2Cap Oxygen analyser, Oxigraf, Mountain View, CA, USA). The pneumotachograph was calibrated using a 3-L syringe (Han-Rudolph 5530, Kansas City, KS, USA) and the gas analysers were calibrated using gas mixtures of known concentrations of O_2 and CO_2 prior to each testing session.

Arterial blood gas variables: A 20-22 gauge arterial catheter was placed into a radial artery and blood samples (2 mL) were taken over approximately 5 cardiac cycle periods. Core body temperature was telemetrically recorded from an ingestion pill (CorTemp, HQInc, Palmetto, FL, USA). All samples were analysed immediately for arterial pH, PO_2 (PaO_2), PCO_2 ($PaCO_2$) (Rapidlab™ 248, Siemens Healthcare Diagnostics Inc., Henkestrasse, Germany), haemoglobin concentration ([Hb]) and O_2 saturation (SaO_2) (Radiometer OSM3, Radiometer Medical ApS, Copenhagen, Denmark). The blood gas values were analysed in triplicate and temperature corrected (26, 53). Arterial bicarbonate concentration ($[HCO_3^-]$) was subsequently calculated using the Henderson-Hasselbalch equation.

Data acquisition

All analog data were sampled and recorded at 200Hz on a PC for off-line analysis (ADInstruments Powerlab 16/30, Bella Vista, Australia).

Data analysis

Steady-state responses

Since the subjects could not tolerate $PETCO_2$ clamping at 50 mmHg at ALT16, the steady-state MCAv-CO_2, MAP-CO_2 and CVCi-CO_2 slopes were estimated from the difference in mean MCAv, MAP and CVCi at the end of 20 and 40 mmHg $PETCO_2$ clamp (20 sec averages), plotted against the change in $PaCO_2$ between these two conditions across all time points (SL, ALT1, ALT16, POST7 and POST21). The absolute value of $\dot{V}E$ at clamp 40 mmHg was used as an estimate of steady-state $\dot{V}E$ responsiveness to CO_2, since voluntary hyperventilation was necessary to reduce $PETCO_2$ to 20 mmHg.

Modified rebreathing

The rebreathing data were first reduced to one-second averages across the entire rebreathing period. The $\dot{V}E$-CO_2 slopes were analysed using a specially-designed programme (Analyse $\dot{V}E$ Rebreathing programme rev11, University of Toronto, Toronto, Canada), as previously described (15, 16, 34). The MCAv-CO_2 slopes were analysed using a commercially available graphing programme (Prism 5.0d, GraphPad Software Inc., San Diego, CA, USA), whereby segmental linear regression (least squares fit) was used to estimate the MCAv-CO_2 slope during the modified rebreathing. For comparison, we plotted the MCAv-CO_2 slopes using a sigmoid curve as described by Battisti-Charbonney et al., (4), using the Prism programme. To minimise the sum of squares for non-linear regression (Levenberg-Marquardt algorithm) we used the equation:

$$\text{MCAv} = a + (b/(1 + \exp(-(\text{PETCO}_2 - c)/d)))$$

Where MCAv is the dependent variable in cm/s, $PETCO_2$ is the independent variable in mmHg, *a* is the minimum MCAv determined from the mean MCAv of the hypocapnic (hyperventilation) region, *b* is the maximum MCAv value, *c* is the mid-point value of MCAv, and *d* is the range of the linear portion of the sigmoid (inverse reflection of the slope of the linear portion).

We found good agreement in the MCAv-CO_2 slope obtained from these two models (R^2=0.71). However, due to the range of $PETCO_2$ used in this study, segmental linear regression generally provided better fit across all conditions, whereas the sigmoidal curve model was the preferred model for only 12 out of 58 trials. As such, only the MCAv-CO_2 slopes obtained using the segmental linear model are presented.

Statistical analysis

Due to logistics impacts on planning and transportation, not all subjects were able to participate in all high-altitude studies, please see the Figures and Table for complete sample size reporting for each procedure. Most data are reported as the improvement over the time of acclimatization (change from ALT1 to ALT16) and as the amount of that improvement that was retained after time at low altitude, calculated as % retention = (POST7 or POST21 – ALT1)/(ALT16 – ALT1)*100 (5). The effects of altitude acclimatisation and re-exposure (between SL, ALT1, ALT16, POST7 and POST21) on the steady-state MCAv-CO_2 slope, CVCi-CO_2 slopes and $\dot{V}E$ at 40 mmHg, were analysed using mixed model linear regression (IBM® SPSS® Statistics version 21, IBM® Corporation, Armonk, NY, USA). To assess the effects of altitude acclimatisation (between SL, ALT1 and ALT16) on the rebreathing estimates of MCAv-CO_2 and $\dot{V}E$-CO_2 slopes, we used mixed model linear regression analysis (Diagonal repeated covariance assumed). The interactions between variables of interest were assessed using correlational (Pearson) analysis (IBM® SPSS®, Statistics version 21). Data are shown as mean ± SD. Results were considered significant at the alpha level <0.05. Trends were consider at the alpha <0.10 level. A priori power calculations ($\alpha = 0.05$, $\beta = 0.20$) were used to determine sample size and limit Type II error.

Results

Detailed baseline characteristics of the 21 (9 females; 21 ± 1 years old) subjects participating in AltitudeOmics are presented elsewhere (54). All 21 subjects completed the protocol at SL. Due to logistical issues, 4 of 21 subjects were unable to complete the entire experimental protocol at ALT1. Upon re-exposure to altitude, 14 of 14 subjects completed the protocol at POST7 and 5 of 7 at POST21. Due to low n, no comparison was carried out between ALT1 and POST21

Resting variables

The resting variables across acclimatisation and re-exposure have already been reported in detail elsewhere (54) and will not be reproduced in this paper.

Steady-state method (Table 1)

Acclimatisation: Compared to SL, the steady-state MCAv-CO_2 slope was elevated at ALT1 (by 79 ± 70%, P<0.001), and was further elevated at ALT16 (by 89 ± 70% vs. ALT1, P=0.001). Similarly, the steady-state MAP-CO_2 slope was elevated at ALT1 (by 256 ± 265%, P=0.013) and further elevated at ALT16 (by 164 ± 1370% vs. ALT1, P<0.001). The steady-state CVCi-CO_2 slope was elevated at ALT1 (by 82 ± 79%, P<0.001), and remained higher at ALT16 (by 93 ± 81%, P<0.001 vs. SL, no difference with ALT1). $\dot{V}E$ at 40 mmHg was elevated at ALT1 compared to SL (by 14.8 ± 12.3 L/min, P=0.011), and further elevated at ALT16 (by 48.3 ± 32.0 L/min vs. ALT1, P<0.001).

Re-exposure: Upon re-exposure to altitude, it appears that the acclimatisation gained in the steady-state MCAv-CO_2 slope was not retained at POST7 (P=0.145 vs. ALT1). Compared to ALT16, the steady-state MCAv-CO_2 slope was lowered at both POST7 and POST21 (P=0.029 & P=0.003, respectively), but nevertheless remained higher compared to SL (P<0.001 & P=0.024, respectively). Similarly, 49% of the acclimatisation gained in the MAP-CO_2 slope was retained at POST7. Specifically, the MAP-CO_2 slope remained higher at POST7 compared to ALT1 (P=0.005). When compared to ALT16, the MAP-CO_2 slope was lowered at both POST7 and POST21 (P<0.001 for both). Nevertheless, MAP-CO_2 slope were higher at POST7 and POST21 compared to SL (P<0.001 & P=0.020, respectively). In contrast, no difference was observed in the CVCi-CO_2 slope at POST7 when compared to ALT1 or ALT16 (P=0.980 & P=0.804, respectively), but it remained higher compared to SL (P<0.001). Likewise, CVCi-CO_2 slope tended to remain higher at POST21 compared to SL (P=0.058), but was not different from ALT16 (P=0.715).

Upon re-exposure, the effect of acclimatisation on the $\dot{V}E$ at 40 mmHg was retained by 38% at POST7 (P=0.004 vs. ALT1). Compared to ALT16, $\dot{V}E$ at 40 mmHg was lower at POST7 and

POST21 (P=0.001 & P<0.001, respectively), but these values remained higher when compared to SL (P<0.001 & P=0.001, respectively).

Modified rebreathing method (Table 1)

Similar to the steady-state method, the rebreathing MCAv-CO_2 slope was elevated at ALT1 (by 137 ± 117%, P<0.001), and further elevated at ALT16 (by 35 ± 33% vs. ALT1, P=0.040). The rebreathing $\dot{V}E$-CO_2 slope was elevated at ALT1 compared to SL (by 1.61 ± 1.14 L/min/mmHg, P=0.038), and further elevated at ALT16 (by 2.86 ± 2.61 L/min/mmHg vs. ALT1, P=0.004). The ventilatory recruitment threshold was lowered at ALT1 (by 4.4 ± 4.0 mmHg, P<0.001 vs. SL) and further lowered at ALT16 (by 4.4 ± 3.2 mmHg vs. ALT1, P<0.001).

Acid-base buffering capacity correlations (Figure 2)

Based on previous findings (16), we performed correlations between the pooled steady-state data with [HCO_3^-] and found resting [HCO_3^-] correlated with steady-state MCAv-CO_2 slope (R=-0.771) and $\dot{V}E$ at 40 mmHg (R=-0.723, P<0.001 for both).

Steady-state vs. modified rebreathing (Figure 3)

We observed correlations between the steady-state and rebreathing MCAv-CO_2 slope at SL (R=0.609, P=0.003), ALT1 (R=0.817, P<0.001) and ALT16 (R=0.596, P=0.007), while the pooled MCAv-CO_2 slopes (combined SL, ALT1 and ALT16) between the two methods also correlated well (R=0.860, P<0.001). Likewise, there were significant correlations between $\dot{V}E$ at 40 mmHg and the rebreathing $\dot{V}E$-CO_2 slope at SL (R=0.476, P=0.029), ALT1 (R=0.506, P=0.038) and ALT16 (R=0.927, P<0.001), while the pooled ventilatory data across all time points were also correlated (R=0.904, P<0.001).

Discussion

The present study is the first to assess the effect of altitude acclimatisation and re-exposure on cerebrovascular CO_2 reactivity using both the steady-state and modified rebreathing methods. We demonstrate that cerebrovascular CO_2 reactivity was elevated immediately upon arrival to 5,260m and is further elevated following 16 days acclimatisation, regardless of the method of assessment. In addition, we found that cerebrovascular and ventilatory responsiveness to CO_2 remains elevated upon re-exposure to altitude, despite 7 or 21 days at low altitude. Since these changes in cerebrovascular and ventilatory responsiveness to CO_2 correlated with the changes in resting arterial [HCO_3^-] across all time points, we speculate that these changes might be partly due to an altered pH buffering capacity associated to exposure high altitude. Our data thus demonstrate that the changes in cerebrovascular and ventilatory control gained due to altitude acclimatisation over a period of 16 days are partially preserved upon subsequent exposure to altitude, at least for up to a period of 3 weeks spent at low altitude.

Effects of acclimatisation on cerebrovascular CO_2 reactivity

Our findings extend those from Fan et al., (16, 17) by demonstrating that the MCAv-CO_2 slope is elevated upon arrival at 5,260 m and further elevated following 16 days of acclimatisation (Fig. 1A). Importantly, previous studies by Fan et al., (16, 17) assessed MCAv-CO_2 slope in subjects whom spent 8 days ascending to 5,050 m, while the subjects in the present study ascended rapidly to altitude (•3 hours), thus making direct comparison difficult. Our findings contradict those of Lucas et al., (30), who found that the MCAv-CO_2 slope was initially elevated at 5,050 m, but had returned towards sea level values following two weeks at 5,050 m. However, because $PETO_2$ was not controlled, the MCAv-CO_2 slopes reported by Lucas et al., (30) reflect MCAv changes from *hypoxic hypocapnia* (room air breathing at 5,050 m; $PETO_2$ •48 mmHg & $PETCO_2$ 26-22 mmHg) to *hypercapnic hyperoxia* ($PETO_2$ > 310 mmHg & $PETCO_2$ •30 mmHg), and thus do not represent isolated reactivity to CO_2. Rupp et al., (49) recently found the MCAv response to steady-state *hypoxic hypercapnia* ($PETO_2$ = 55 mmHg) to be reduced following 5 days at 4,350 m. Therefore, discrepancies between findings Rupp et al., (49) and those of the present study can be attributed the differences in $PETO_2$ (55 mmHg vs. >200 mmHg), altitude (4,350 m vs. 5,260 m), and the acclimatisation state of the subjects (5 days vs. 16 days). The results from the present study demonstrate, for the first time, that cerebrovascular CO_2 reactivity *per se* is enhanced with acclimatisation to high altitude when studied using a background level of hyperoxia. Furthermore, discrepancy between studies highlights how methodological differences can yield vastly different results. Thus future studies are warranted to clarify the effect of hypoxic and hyperoxic background on assessing cerebrovascular functions at both sea-level and following ascent to high altitude.

Altered acid-base buffering capacity?

During altitude acclimatisation, there is a progressive and parallel reduction in arterial and cerebrospinal fluid (CSF) bicarbonate concentration which serves to compensate for the changes in pH associated with hyperventilation-induced hypocapnia (12, 13, 20). These changes in acid-base buffering capacity, in both the arterial and CSF compartments, would lead to a greater rise in arterial and CSF [H^+] for a given rise in $PaCO_2$. In support of this notion, lowering CSF bicarbonate concentration elevates the cerebrovascular CO_2 reactivity in an anaesthetised dog model (27), while bicarbonate infusion increases cerebral perfusion pressure in post-traumatic head injury patients (9), elevates cerebral blood volume in preterm infants (57), and lowers ventilation in healthy exercising humans at sea-level (44). As such, it has been suggested that the MCAv responses to CO_2 at high altitude are linked to changes in arterial acid-base balance (16, 25). In the present study, we observed concomitant increases in cerebrovascular and ventilatory responsiveness to CO_2 with acclimatisation to high altitude and re-exposure (Fig. 1), which occurred in parallel to the changes in [HCO_3^-] (Fig. 2). While it should be acknowledged that such correlations do not imply causality, the possible role for acid-base status changes on cerebrovascular and ventilatory responsiveness to CO_2 at high altitude remains to be further studied.

Interaction between cerebrovascular and ventilatory responsiveness to CO_2

Interaction between cerebrovascular CO_2 reactivity and the central chemoreceptor activation was first alluded to by Heyman et al., (22) and has been subsequently expanded upon by others (10, 16-18, 38, 43, 60-62). It was postulated that changes in cerebrovascular CO_2 reactivity affect the stability of ventilatory response to CO_2 by modulating the degree of H^+ washout at the level of the central chemoreceptor (38). Accordingly, a blunted cerebrovascular CO_2 reactivity would lead to less central H^+ washout and subsequently greater central chemoreceptor activation. Conversely, an enhanced cerebrovascular CO_2 reactivity would result in lower central [H^+] and therefore lower ventilatory CO_2 sensitivity. In agreement with previous altitude studies (16, 17), we observed concomitant increases cerebrovascular and ventilatory responsiveness to CO_2 (Fig. 1). These findings seem to contradict the modulating role of cerebrovascular CO_2 reactivity on central chemoreceptor activation, possibly due to other overriding factors such as enhanced central chemosensitivity and changes in acid-base balance associated with ascent to high altitude. Future work is necessary to further unravel the interaction between the regulation of cerebral blood flow and ventilation.

Going back up

Despite the large body of literature regarding high altitude acclimatisation over the past century, the effect of prior exposure on physiological parameters during subsequent exposures is not well documented. Most attention focused on the effect of a recent altitude exposure on the risk for AMS (7, 31, 45, 51), or the rate of ascent (56). However, the dose of previous altitude exposure and acclimatisation were generally not controlled in these studies. Wu et al., (59) found a progressive reduction in the incidence of AMS, lower HR and higher SpO_2 in lowland railroad workers over the course of several seven-month exposures to high altitude interspersed with 5 months spent at low altitude. Similarly, MacNutt et al., (32) found faster rate of ascent, lower AMS and higher SpO_2 in trekkers with a recent altitude exposure compared to altitude naive trekkers, despite a 7-30 day de-acclimatisation period. In the present study, we compared the cerebrovascular and ventilatory responsiveness to CO_2 with acclimatisation and upon re-exposure to 5,260 m following a period of either 7 or 21 days at low altitude. We found that 38% of the gain in ventilatory response to CO_2 over acclimatisation was retained at POST7 (Fig. 1C), while essentially none of the gain in MCAv-CO_2 reactivity over acclimatisation was retained at POST7 (Fig. 1A). Regardless of the underpinning mechanism(s), our findings suggest that the effect of previous altitude acclimatisation over 16 days on ventilatory response to CO_2 is partially retained after 7 days at low altitude, while it is reversed in the cerebrovascular response to CO_2. Our data extends those by Muza et al., (36) which showed that ventilatory acclimatisation gained at 4,300 m is retained following 8 days spent at low altitude. Since we found the CVCi-CO_2 slope to be consistently elevated by 60-80% across all time points (Fig. 1D), while the changes MAP-CO_2 slope closely follows the changes in MCAv-CO_2 slopes (Fig. 1B), we speculate that the changes in MCAv-CO_2 slopes at high altitude can be primarily accounted for by an enhanced sensitivity of the cerebral vessels to CO_2, whereas the remainder can be attributed to an enhanced perfusion pressure response.

Steady-state or modified rebreathing method?

There has been much debate over the use of the steady-state or the modified rebreathing method for the assessment of cerebrovascular and ventilatory control, and attempts at consensus have produced no uniform agreement [(18, 40), also see (2, 14) for reviews]. The steady-state ventilatory responses to CO_2 were found to be either similar (34, 37, 40-42, 47) or lower (6, 18, 23, 55) when compared to rebreathing estimates., while steady-state cerebrovascular CO_2 reactivity has been shown to be consistently higher than rebreathing values (18, 40-42). The present study demonstrates that the changes in cerebrovascular and ventilatory CO_2 responsiveness with altitude acclimatisation were similar between the steady-state and the modified rebreathing method (Table 1) – possibly due to tight control of arterial PCO_2 and PO_2 with our end-tidal clamping setup. Moreover, we observed strong correlations in these parameters between the two methods across all time points (Fig. 3). We therefore conclude that both methods can be used to assess the changes in cerebrovascular and ventilatory responses to CO_2 with high altitude exposure and acclimatisation, provided that the level of CO_2 is comparable across all the conditions, under identical level of background O_2.

Limitations

Although the present study provided the opportunity to assess the effects of acclimatisation and re-exposure to 5,260 m on the cerebrovascular CO_2 reactivity, an important methodological consideration should be acknowledged when interpreting our findings. In the present study, transcranial Doppler ultrasound (TCD) was used to measure the MCAv, as an index of global CBF changes during initial exposure, acclimatisation and subsequent re-exposure to 5,260 m. This is based on the assumption that: i) the MCA carries approximately upwards of 80% of the overall blood flow to the respective hemisphere (29); ii) changes in MCAv reflect changes in global CBF (8, 52); iii) the changes in MCAv in response to $PaCO_2$ changes are comparable to the changes of internal carotid blood flow (50); and iii) the diameter of the MCA does not change during the observed changes in arterial blood gases (52). In support, MCAv has been shown to reflect changes in CBF assessed with the direct Fick method, at least during initial exposure to high altitude (33, 35, 48).

Recent findings by Wilson et al., (58) indicate that the diameter of MCA, as measured using TCD, vary, depending on the altitude (e.g., 5.30 mm at 75 m, 5.51 mm at 3,500 m, 5.23 mm at 5,300 m and 9.34 mm at 7,950 m). Importantly, the results from Wilson et al., (58) demonstrate that the MCA diameter remains relatively unchanged up to 5,300 m. It should be noted that the MCA diameters measured with TCD in that study were 80-90% greater than the values obtained using magnetic resonance imaging in the same subjects. Since our measurements were carried out in background hyperoxia ($PETCO_2$ > 300 mmHg), it seems unlikely that our cerebral blood velocity values would be confounded by any effect of hypoxia-induced vasodilation of the MCA. Further studies are needed to evaluate MCAv responses to CO_2 while holding $PETO_2$ at consistent levels of hypoxia.

Conclusion

Findings from the present study clearly show that both cerebrovascular and ventilatory responsiveness to CO_2 is elevated upon arrival at high altitude and further elevated with acclimatisation. We demonstrate, for the first time, that this effect of high altitude acclimatisation on the ventilatory response to CO_2 is partially retained after a period at low altitude, while prior acclimatisation has no effect of the cerebrovascular response to CO_2. Our data suggest that the increased cerebrovascular CO_2 reactivity with acclimatisation may be accounted for by the changes in acid-base balance in the blood and possibly the cerebrospinal fluid compartment.

Grants

This study was supported by the Swiss National Science Foundation and the Faculty of Medicine of the University of Geneva. The overall AltitudeOmics study was funded, in part, by grants from the U.S. Department of Defense (W81XWH-11-2-0040 TATRC to RR, and W81XWH-10-2-0114 to ATL); the Cardiopulmonary & Respiratory Physiology Laboratory, University of Oregon; the Altitude Research Center and the Charles S. Houston Endowed Professorship, Department of Emergency Medicine, School of Medicine, University of Colorado Denver.

Author contributions

JF contributed to conception and design of the experiments, data collection, data analysis, data interpretation, manuscript drafting and editorial process. OE contributed to the design of the experiments, data collection, data analysis and manuscript revision. NB contributed to the data collection and the manuscript revision. BK contributed to the interpretation of the data and the revision of the manuscript. AL, AS and RR conceived, designed and executed the AltitudeOmics study of which the present study was a part, and contributed to manuscript revision. AS also contributed to data collection and data interpretation. All authors approved the final version of the manuscript.

Acknowledgement

This paper is part of a series titled "AltitudeOmics" that together represent a group of studies that explored the basic mechanisms controlling human acclimatisation to hypoxia and its subsequent retention. Many people and organisations invested enormous amounts of time and resources to make AltitudeOmics a success. Foremost, the study was made possible by the tireless support, generosity and tenacity of our research subjects. AltitudeOmics principal investigators were Colleen G. Julian, Andrew T. Lovering, Andrew W. Subudhi and Robert C. Roach. A complete list of other investigators on this multinational, collaborative effort involved in development, subject management and data collection, supporting industry partners, and people and organisations in Bolivia that made AltitudeOmics possible is available in the first paper in this series (54). The authors are extremely grateful to Julia Kern, Jon E. Elliot, Steve S. Laurie and Kara M. Beasley for their invaluable assistance in the blood gas data collection for this study. We extend our gratitude to Prof. James Duffin, who kindly provided his assistance and the rebreathing analysis program. We would like to thank Roberto Molinari for his assistance in the statistical analysis of the data.

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Table 1. Cerebrovascular and ventilatory reactivities parameters during the steady-state and modified rebreathing (mean ± SD).

	SL (n=21)	ALT1 (n=17)	ALT16 (n=20)	POST7 (n=14)	POST21 (n=5)
Steady-state					
MCAv-$PaCO_2$ slope (cm/s/mmHg)	1.19 ± 0.42	2.16 ± 1.05*	3.39 ± 0.89*†	2.68 ± 0.88*§	2.06 ± 0.57*§
CVCi-$PaCO_2$ slope (%/mmHg)	3.35 ± 1.21	5.87 ± 2.60*	5.75 ± 1.85*	5.89 ± 1.23*	5.41 ± 1.78*
MAP-$PaCO_2$ slope (L/min)	0.03 ± 0.24	0.28 ± 0.19*	1.06 ± 0.45*†	0.56 ± 0.29*§	0.32 ± 0.18*§
$\dot{V}E$ at 40 mmHg (L/min)	19.15 ± 4.89	34.06 ± 12.23*	80.05 ± 32.32*†	49.03 ± 13.68*§†	43.25 ± 7.56*§
Modified rebreathing					
MCAv-$PETCO_2$ slope (cm/s/mmHg)	1.34 ± 0.60	2.95 ± 1.11*	3.67 ± 0.87*†	-	-
$\dot{V}E$-CO_2 slope (L/min/mmHg)	1.90 ± 0.81	3.49 ± 1.51*	6.28 ± 3.56*†	-	-
$\dot{V}E$ recruitment threshold (mmHg)	38.7 ± 3.4	33.7 ± 3.7*	29.2 ± 2.1*†	-	-

* different from SL ($P<0.05$); † different from ALT1 ($P<0.05$); § different from ALT16 ($P<0.05$).

Figure legend

Figure 1 Changes in steady-state estimates of cerebrovascular, cardiovascular and ventilatory responsiveness to CO_2 with acclimatisation and re-exposure to 5,260 m. Values expressed as mean ± SD. * different from SL (P<0.05), † different from ALT1 (P<0.05), § different from ALT16 (P<0.05).

Figure 2 Relationship between standard basic excess and steady-state cerebrovascular, ventilatory and cardiovascular responsiveness to CO_2 with acclimatisation to altitude. * significant correlations (P<0.05).

Figure 3 Comparison of steady-state and rebreathing estimates of cerebrovascular and ventilatory responsiveness of CO_2 with acclimatisation to 5,260 m. * significant correlations (P<0.05).

Figure 1

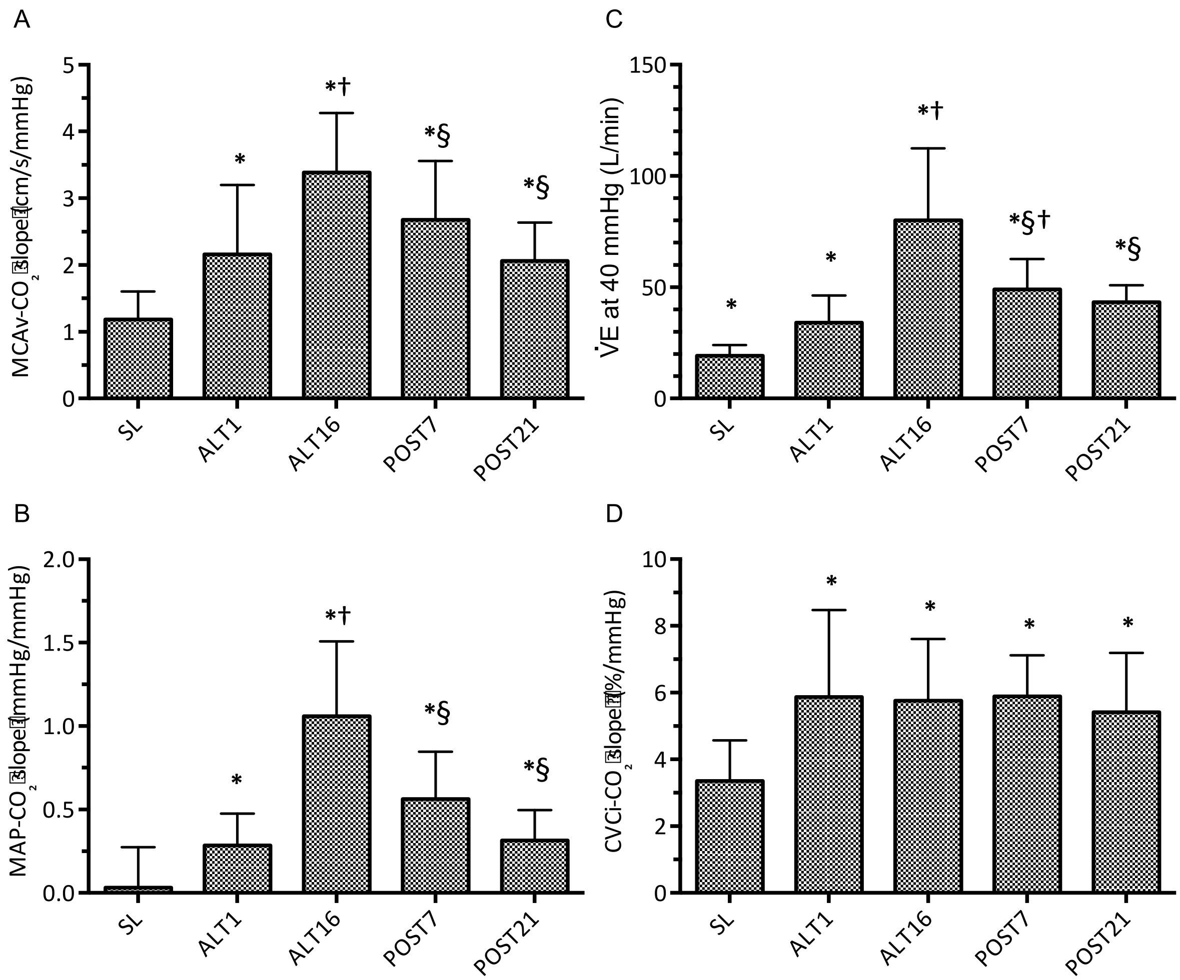

A
MCAv-CO2 slope (cm/s/mmHg)
C
V̇E at 40 mmHg (L/min)
B
MAP-CO2 slope (mmHg/mmHg)
D
CVCi-CO2 slope (%/mmHg)
SL
ALT1
ALT16
POST7
POST21
*
*†
*§
*§†

Figure 2

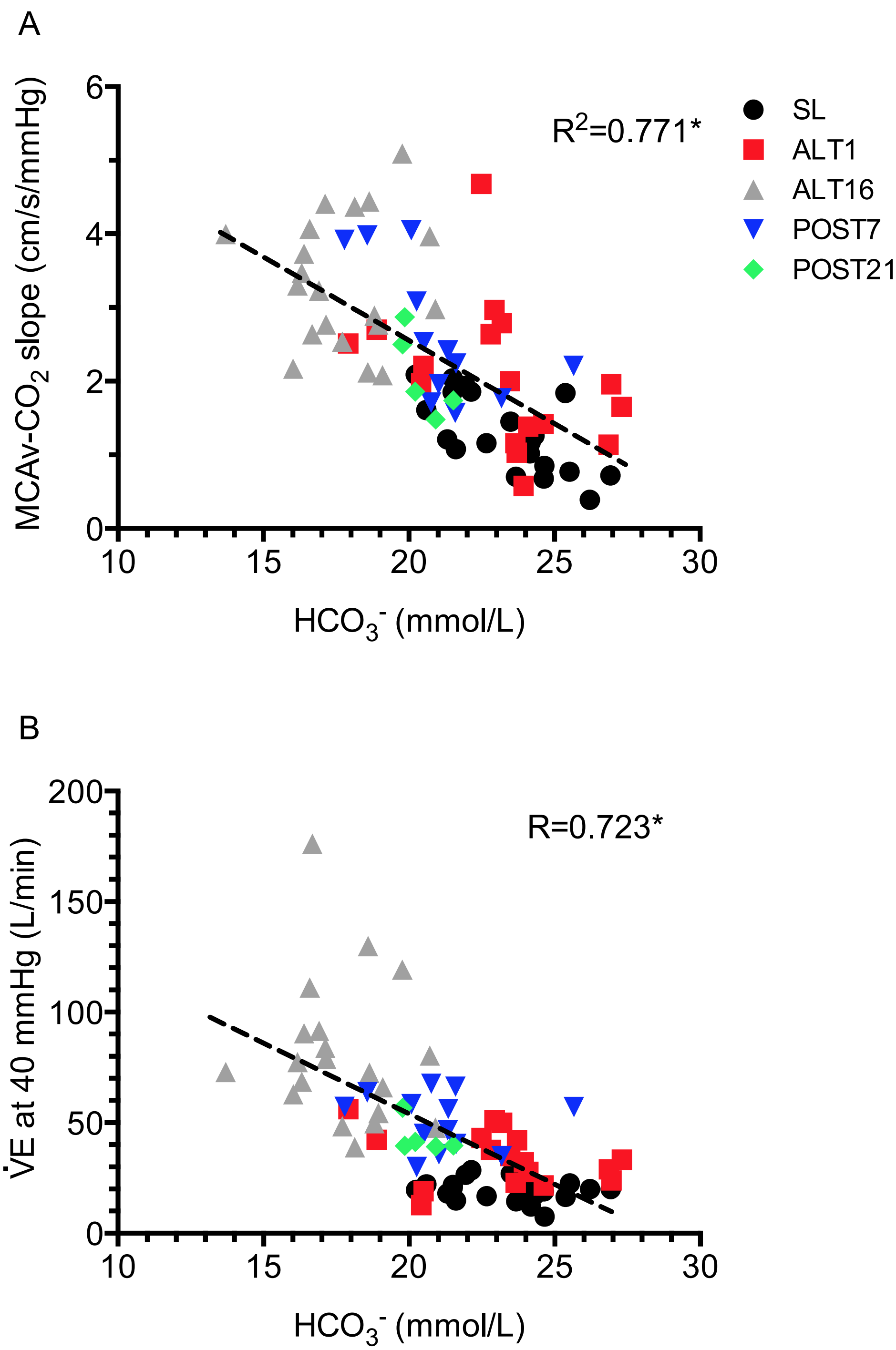
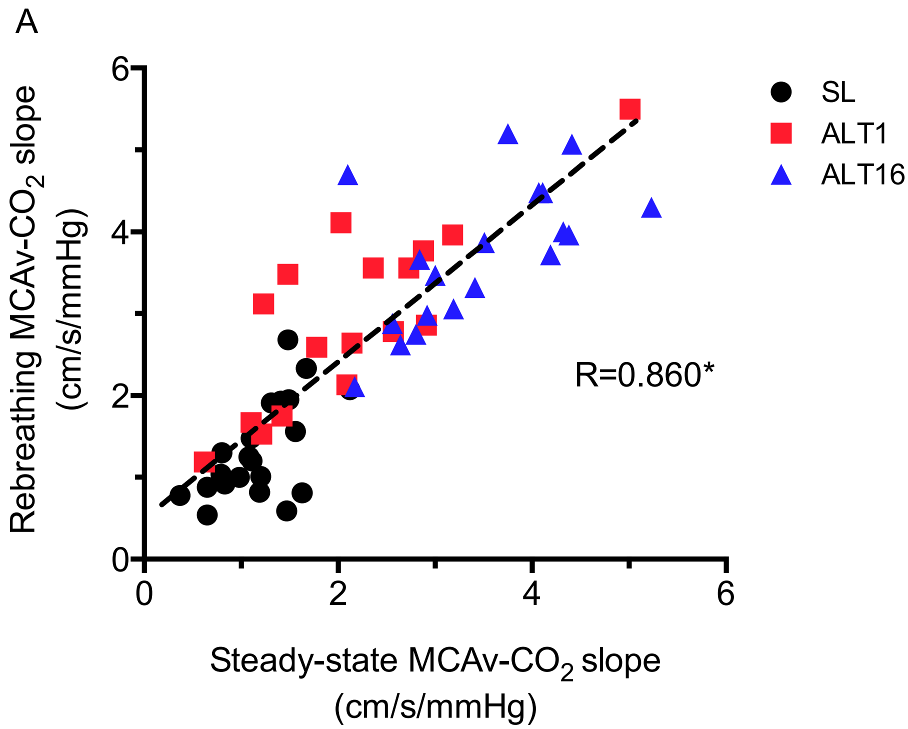
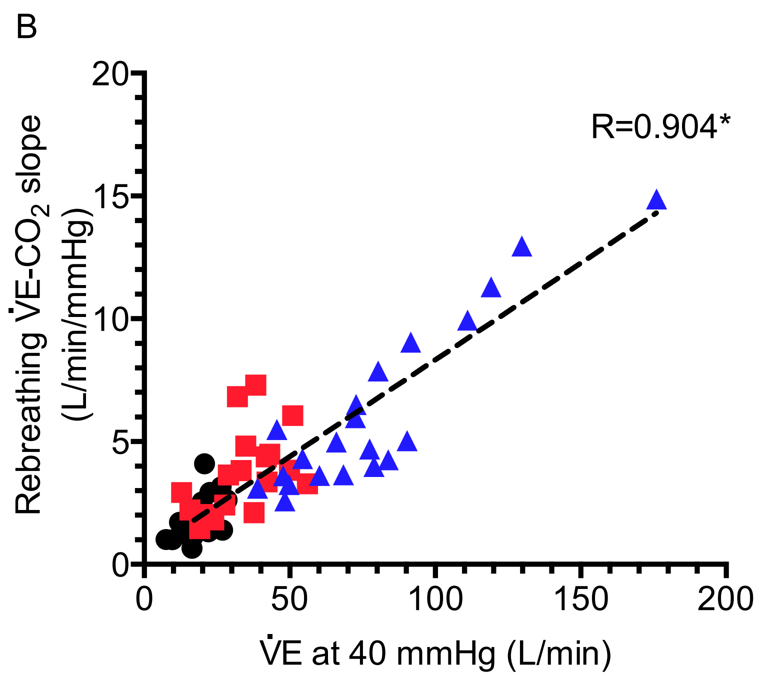

A
MCAv-CO2 slope (cm/s/mmHg)
R2=0.771*
SL
ALT1
ALT16
POST7
POST21
0
2
4
6
10
15
20
25
30
HCO3- (mmol/L)
B
V̇E at 40 mmHg (L/min)
R=0.723*
0
50
100
150
200
10
15
20
25
30
HCO3- (mmol/L)

Figure 3



A
Rebreathing MCAv-CO_2 slope (cm/s/mmHg)
Steady-state MCAv-CO_2 slope (cm/s/mmHg)
0
2
4
6
SL
ALT1
ALT16
R=0.860*



B
Rebreathing V̇E-CO_2 slope (L/min/mmHg)
V̇E at 40 mmHg (L/min)
0
5
10
15
20
50
100
150
200
R=0.904*

Articles in PresS. J Appl Physiol (December 26, 2013). doi:10.1152/japplphysiol.00880.2013

AltitudeOmics: Cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness

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Running Head: Cerebral autoregulation at altitude

Key Words: transcranial Doppler, cerebral blood flow, cerebral oxygenation, transfer function analysis, hypoxia

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Abstract

Cerebral autoregulation (CA) acts to maintain brain blood flow despite fluctuations in perfusion pressure. Acute hypoxia is thought to impair CA, but it is unclear if CA is affected by acclimatization or related to the development of acute mountain sickness (AMS). We assessed changes in CA using transfer function analysis of spontaneous fluctuations in radial artery blood pressure (indwelling catheter) and resulting changes in middle cerebral artery blood flow velocity (transcranial Doppler) in 21 active individuals at sea level (SL), upon arrival at 5,260 m (ALT1), after 16 days of acclimatization (ALT16), and upon re-exposure to 5,260m after 7 days at 1,525 m (POST7). The Lake Louise Questionnaire (LLQ) was used to evaluate AMS symptom severity. CA was impaired upon arrival at ALT1 ($P<0.001$) and did not change with acclimatization at ALT16 or upon re-exposure at POST7. CA was not associated with AMS symptoms (all $R < 0.50$, $P > 0.05$). These findings suggest that alterations in CA are an intrinsic consequence of hypoxia and are not directly related to the occurrence or severity of AMS.

Introduction

Cerebral autoregulation (CA) is a general term used to describe dynamic myogenic, neurologic and metabolic responses that adjust cerebrovascular resistance to maintain relatively constant cerebral blood flow across a wide range of perfusion pressures (25). Dynamic CA is said to be impaired if fluctuations in mean arterial blood pressure lead to concurrent fluctuations in mean cerebral blood flow. Impairments in CA are associated with cerebrovascular disorders (3, 24, 31), yet the relative importance of CA in the development and course of certain pathologies is unclear.

Our initial interest in CA stemmed from the hypothesis that impaired CA may be involved in the development of acute mountain sickness (AMS), high-altitude headache and cerebral edema (5, 7, 9, 16, 37). Conversely, we showed that impairments in CA upon acute exposure to hypobaric hypoxia preceded, but were not associated with, the development of AMS (2, 33, 35). Furthermore, since several cross-sectional studies demonstrated that impairments in CA persist from 1 to 30 days of high-altitude exposure (1, 2, 11, 12, 17) - when AMS is not present – and are evident in healthy, permanent high-altitude residents (12, 13), it seems reasonable to suggest that a shift in CA may be an inherent and relatively benign consequence of hypoxemia.

To date, no longitudinal studies have characterized CA and tested its relation with AMS during acute and chronic high-altitude exposures. Previous studies have either omitted CA measurements upon arrival at high altitude (7, 11, 17), or followed slow ascent profiles that allow for partial acclimatization prior to initial measurements (1, 12, 39). In this study, we present novel data from sea-level residents who rapidly ascended to high altitude (5,260 m), acclimatized for 16 days, and were subsequently re-exposed to high altitude after spending 7 days at low altitude (1,525 m). Specifically, we tested the hypotheses that CA would be: 1) impaired upon rapid ascent to high altitude, 2) unaffected by 16 days of acclimatization, 3) unaffected upon re-exposure to the same altitude, and 4) unrelated to the occurrence or severity of AMS.

Methods

Study overview

This study was conducted as part of the AltitudeOmics project. Briefly, institutional ethics approval was obtained from the Universities of Colorado and Oregon and the US Department of Defense Human Research Protection Office. Young, healthy sea-level residents were recruited from the greater Eugene, Oregon area (elevation 128 m) and screened to exclude anyone who was born or had lived at altitudes >1,500 m for more than one year or had traveled to altitudes > 1,000 m in the past 3 months. After obtaining written consent, physical exams and the Army Physical Fitness Test (push ups, sit ups and 3.2 km run) were performed to verify health and fitness status. Approximately 4 weeks following sea-level (SL) measurements in Eugene, Oregon, subjects were flown to La Paz, Bolivia. They spent two nights at low altitude (Coroico, Bolivia, 1,525 m) before being driven to the Chacaltaya research station at 5,260 m while breathing supplemental oxygen. Acute responses to high altitude were assessed ~4 hours after arrival and cessation of supplemental oxygen (ALT1). Subjects acclimatized to altitudes ranging from 3,800 to 5,260 m over the next 15 days, with most of the time (75%) spent at 5,250 m. On the 16th day (ALT16), measurements were repeated at 5,260 m before subjects were driven down to Coroico for either 7 or 21 days. Subjects were driven back to the laboratory at 5,260 m for POST 7 or POST 21 re-exposure measurements.

This report focuses on novel data regarding resting CA evaluated immediately prior to a series of cerebrovascular, respiratory and exercise interventions, as outlined elsewhere (32). We have carefully avoided replication of data between reports, except where common variables were necessary to describe subjects' basic physiologic status at the time points of interest (e.g. heart rate, blood pressure, arterial blood gases).

Physiology Protocol

All subjects were familiarized with study procedures during a practice session at least 48 hours before experimental testing at SL. Subjects followed standardized exercise and dietary regimens for 24 hours prior to each measurement period. At each time point, a 22-gauge catheter was inserted into a radial artery at least 1 hour prior to instrumentation. Subjects were seated in an upright position for 15 min while sensors were placed to measure physiologic variables of interest. Limb lead electrodes were used to measure ECG (BioAmp, ADInstruments, Colorado Springs, CO, USA). Arterial blood pressure (ABP) was monitored via a fluid filled pressure transducer (Deltran II, Utah Medical, Salt Lake City, UT, USA) attached to the radial artery catheter. Core temperature was telemetrically recorded from an ingested pill (CorTemp, HQInc, Palmetto, FL, USA). Cerebral blood flow velocity (CBFv) in the left middle cerebral artery was measured by transcranial Doppler (2MHz Spencer Technologies, Seattle USA) at depths ranging from 43 to 54 mm. Signal quality was optimized and an M-mode screen shot was recorded to facilitate subsequent probe placements and insonation angles.

After verification of signal quality, resting data were recorded for 6 min while subjects breathed room air to assess CA at each altitude. Continuous analog data (ABP, CBFv, ECG, O_2 and CO_2) were recorded at 200 Hz (ADInstruments Powerlab 16/30, Colorado Springs, CO, USA) for offline analysis. Core temperature and arterial blood samples (2 ml) were taken during the last 30 s of measurement periods. Blood samples were taken from the radial artery catheter and blood gases were analyzed for $PaCO_2$ and PaO_2 in triplicate (RAPIDLab 248, Siemens, Erlangen, Germany) and corrected for body temperature (15, 29).

Acute Mountain Sickness

Self reported sections of the Lake Louise Questionnaire (LLQ) were used to assess AMS on ALT1 and POST7 (~12 hours after arrival). Moderate and severe AMS were defined as LLQ ≥ 3 and ≥ 6, including headache, respectively (27).

Data Analysis

Transfer function analyses were used to assess dynamic CA, based on spontaneous fluctuations in the raw ABP and CBFv signals, as previously described (33, 34). Briefly, 6-min recordings of instantaneous ABP and CBFv were reduced to beat-by-beat averages, resampled at 5 Hz and transformed from the time to frequency domain using fast Fourier transformations (512 points per segment with 40% overlap). The transfer function from mean ABP to CBFv was expressed in terms of coherence, gain, and phase shift in the very low frequency range (0.02 - 0.07 Hz), where dynamic CA is most active (21, 22), as well as in low (0.07 to 0.20 Hz) and high (0.20 to 0.35 Hz) frequency ranges. All data were used in subsequent statistical analyses. Reduction in phase shift was considered the primary criterion for impaired CA because it signifies shorter delay in transmission of pressure (ABP) into flow (CBFv), or a reduction in the ability of the cerebrovascular system to buffer changes in ABP and maintain consistent blood flow. Yet, since increases in gain (increase in CBFv relative to a change in ABP) and coherence (linear correlation between ABP and CBFv) may also suggest CA impairment (8, 24, 41), all three transfer function metrics are reported. To address difficulties in interpreting possible permutations of these three variables, the inverse transfer function of the resulting gain and phase shift was used to express results in the time domain as a step function that could be fitted to one of 10 curves representing a single autoregulation index (ARI) score (36). An ARI score of 0 indicates complete lack of autoregulation and 9 indicates perfect autoregulation.

Statistics

After calculating descriptive statistics (mean ± SD) and verifying normality (D'Agostino and Pearson Test), variables were analyzed by repeated measures ANOVAs to evaluate the effect of time on CA metrics with Fisher's LSD *post hoc* tests and the Holm procedure to correct for multiple comparisons ($\alpha = 0.05$).

Spearman rho correlations were run to evaluate relations between CA metrics and the severity of LLQ symptom scores. Specifically, we tested the ability of CA assessments, measured at SL and upon arrival at ALT1, to predict ensuing symptoms of AMS (7). Also, because AMS classification is dichotomous (i.e. positive vs. negative), we used receiver operating characteristic (ROC) analyses (14, 18) to evaluate the sensitivity (true positive rate) and specificity (true negative rate) of ARI scores' ability to detect mild and severe AMS. The ROC area under the curve (AUC) statistic was used as an indicator of test accuracy. An AUC of 1.0 signifies a perfect test, with no chance of false positive or false negative results, while an AUC of 0.5 signifies a meaningless test, where the probability of identifying a true positive result is only 50%.

Results

Subjects

We studied 21 subjects at SL (12 males and 9 females; 21 ± 1 years old). Because of logistical problems upon arrival in Bolivia, complete data sets were not obtained on the first 7 subjects upon arrival at ALT1. Since the first 7 subjects comprised the cohort studied at POST21, longitudinal assessments of CA were limited to the remaining 14 subjects who completed the study at POST7.

Effect of Rapid Ascent to High Altitude

At SL, resting cardiovascular (HR, ABP, CBFv) and CA measurements (coherence, gain, phase shift and ARI scores) were characteristic of young, healthy individuals with intact CA (Table 1, Figure 1). From SL to ALT1, PaO_2 and $PaCO_2$ decreased (65 and 26%, respectively, $P < 0.001$, Table 1). This degree of hypoxia increased HR ($P < 0.001$), but did not affect mean ABP or CBFv. Very low frequency power spectral density (PSD) of ABP and CBFv were unaltered, but increases in transfer function coherence (P < 0.001) and decreases in phase shift (P < 0.050) and ARI score (P < 0.001) were consistent (in 13 of 14 subjects) with the definition of impaired CA at ALT1.

Effect of Acclimatization to High Altitude

Acclimatization increased resting PaO_2 (27%) and decreased $PaCO_2$ (22%) from ALT1 to ALT16 (both P<0.001), without affecting HR, ABP or CBFv. Measures of CA at ALT16 were unchanged from ALT1 and remained impaired relative to SL in the very low frequency range (all P < 0.010, Table 1, Figure 1).

Effect of Re-exposure to High Altitude

Resting PaO_2 and $PaCO_2$ at POST7 fell between ALT1 and ALT16 values (all P<0.050 vs. ALT1 and vs. ALT16), indicating that the degree of acclimatization achieved at ALT16 was partially maintained at POST7. Assessments of CA at POST7 were similar to those at ALT1 and ALT16 and remained impaired relative to SL in the very low frequency range (P < 0.050, Table 1, Figure 1).

Association between CA and AMS

Of the 21 subjects, 17 reported symptoms of at least moderate AMS at ALT1 (LLQ = 6.4 ± 2.2), 10 of who met the criteria for severe AMS (LLQ = 7.8 ± 1.7). Correlations between CA metrics preceding the development of AMS symptom were weak (all r<0.50, P>0.050, Figure 2). The ROC analysis revealed that ARI scores measured at

SL were not sensitive or specific predictors of moderate (AUC=0.54, P=0.788) or severe AMS (AUC=0.69, P=0.139). Additionally, the degree of impairment in CA (measured as the change in ARI from SL to ALT1) was not a sensitive or specific predictor of moderate (AUC=0.53, P=0.881) or severe AMS (AUC=0.72, P=0.124). None of the 14 subjects studied at POST7 reported symptoms of AMS, thus associations with CA could not be tested.

Discussion

The key findings of this study were that cerebral autoregulation, as assessed by transfer function analysis, is 1) impaired upon rapid ascent to high altitude, 2) unaffected by acclimatization, or 3) subsequent re-exposure to the same altitude, and 4) not a sensitive or specific predictor of AMS. Based on our results we question whether the so-called impairment in CA that persists at high altitude is characteristic of pathological insufficiency in cerebrovascular regulation (16), or alternatively reflects a relatively benign relaxation in autoregulation.

Effect of high altitude on CA

This is the first longitudinal study of CA at high altitude, from rapid ascent through acclimatization and upon re-exposure after a short period at low altitude. We show that impairment of CA was a consistent characteristic across this high-altitude exposure profile.

Increased transfer function coherence and gain along with reduced phase shift and ARI score upon rapid ascent were all consistent with the classic definition of impaired CA (Table 1) and outside the normal range of expected variability (6), implying that changes in ABP were more readily transmitted into the cerebral circulation as changes in CBFv at high altitude. Our finding of impaired CA after less than one day of travel from low to high elevation is consistent with our previous findings after 4 hours in a hypobaric chamber (35) and fills an important gap in the literature between studies conducted in laboratories with hypoxic gas mixtures, where normobaric hypoxia was achieved in a matter of minutes (5, 10, 26, 34), and studies of trekkers, where several days of progressive ascent preceded initial high-altitude measurements (1, 2, 12, 37). Impaired CA at rest in acute hypoxia is a consistent finding among all but one study (26), suggesting that neither the mode nor rate of ascent appears to affect the general assessment.

By evaluating CA upon initial exposure and after 16 days at high altitude, we were able to determine if changes in CA occur with acclimatization, as might be expected with increased PaO_2 (2, 35), decreased $PaCO_2$ (19, 23, 26), and further sympathoexcitation (1). On the contrary, we found no change in CA over the course of acclimatization (Table 1). Our longitudinal findings are consistent with other cross-sectional studies demonstrating impaired CA at various time points after arrival at high altitude (1, 2, 7, 11, 12, 37) and in permanent high-altitude residents (12, 13). These results may indicate that assessments of CA are less sensitive to changes in PaO_2 and $PaCO_2$ near their respective extremes. Alternatively, a slight improvement in CA due to increased PaO_2 (2, 35) may have been masked if the opposing effects of $PaCO_2$ (19, 23, 26) and/or sympathoexcitation (1) on CA were heightened over time at altitude. Further testing with manipulation of arterial gases and sympathetic activity is necessary to determine the relative influence of arterial gases and neural stimulation on CA at high altitude, yet impaired CA remains a consistent functional consequence across time at high altitude.

As an additional test of the hypothesis that impaired CA is a consistent response to hypoxemia, we sent subjects down to low altitude for 7 days and re-evaluated their CA response after a second rapid ascent back to high altitude. Upon re-exposure, the measured impairment in CA was similar to that observed upon the first ascent (ALT1) and after acclimatization (ALT16). Together, these results demonstrate that impaired autoregulation was a consistent characteristic of hypoxemia across our study and imply that slow fluctuations in arterial pressure were less effectively dampened by the cerebral vasculature regardless of the state of acclimatization. What remains to be determined is if such a tenuous pressure-flow relation may be potentially harmful.

Relation of CA to AMS

Impairment of CA has been suggested to play a role in the development of AMS by either permitting cerebral overperfusion and mechanical disruption of the blood brain barrier (i.e. vasogenic cerebral edema) when mean ABP is elevated, or by cerebral under-perfusion and exacerbation of cerebral hypoxia/ischemia when mean ABP is lowered (9, 16). In the present study, we found no correlation between measures of CA and subsequent AMS symptom scores (Figure 2), which opposes the notion that lower CA predisposes people to AMS, or conversely, that higher CA confers protection from AMS. Our additional ROC analyses of AMS status, confirmed that ARI scores were neither sensitive nor specific indicators for the development of moderate or severe AMS upon arrival at high altitude. These findings are congruent with our previous report following the time course of changes in CA and AMS symptoms over the first 10 hours of exposure to hypobaric hypoxia (35), where we found similar levels of CA impairments in subjects who eventually developed AMS or stayed healthy, but are at odds with other studies showing some association between CA and AMS symptoms (5, 37). Our data also counter a recent finding that sea-level assessments of CA predict ensuing severity of AMS (7).

Discrepancies between studies may be explained by the various methods used to assess CA (transfer function vs. leg cuff – see Limitations), the questionnaires used to assess AMS (LLQ vs. Environmental Symptoms Questionnaire), and the statistical approach used to evaluate the relation between CA and AMS (correlation vs. ROC). We acknowledge that caution should be exercised when interpreting correlations with an ordinal level variable, such as the LLQ score, because by definition the scale has limited mathematical meaning. For example, an LLQ score of 6 does not imply symptom severity is exactly twice that of a score of 3. Due to the intrinsic level of measurement, we believe that LLQ scores are best restricted to dichotomous classification of positive or negative AMS status, and thus place more emphasis of the negative results of our ROC analysis. We encourage others to consider this method of analysis for future AMS studies.

Overall, given the similarity in CA responses among individuals with a wide range of AMS scores, we do not believe that changes in CA cause AMS. This assertion is further supported by the complete lack of association between impaired CA at POST7 when no symptoms of AMS were reported and previous reports documenting impaired CA in healthy high-altitude natives (12, 13). Nonetheless, we must acknowledge that the alteration in CA upon acute altitude exposure may set up a tenuous pressure-flow relation which could permit AMS to develop, if other, yet unidentified, factors are present at the same time.

Since impairment of CA appears to be a consistent physiological response in hypoxic environments and unrelated to AMS status, it is tempting to speculate that the underlying change in the cerebral pressure-flow relation may actually promote successful acclimatization or adaptation to chronic states of hypoxemia (4). It is possible that impairment of CA could promote cerebral oxygen delivery in a time of need since it allows greater cerebral perfusion for a given increase in ABP. This potentially beneficial consequence of impaired CA during hypoxemic stress might outweigh the relative risk of reduced cerebral perfusion if ABP were to drop. We therefore raise the possibility that the term impaired CA may be a misnomer because it implies an association with pathology that has yet to be substantiated in acute or chronic hypoxemia. We suggest that relaxation of CA might be a more accurate term to describe changes in the cerebral pressure-flow relation from normoxia to hypoxia in the absence of pathology.

Limitations

One major limitation affecting the field is the lack of a gold standard method to assess CA. We have chosen to evaluate rhythmical fluctuations in CA via transfer function analysis, primarily because we believe it captures the natural cerebral pressure-flow relation over time and thus has greater practical relevance over methods which induce larger, more abrupt changes in ABP, as with leg cuff inflation/deflation, rapid tilting, or more sustained changes in ABP, as with pharmaceutical interventions. Still, we acknowledge that transfer function analysis of resting data monitors relatively subtle fluctuations in ABP and CBFv, which, if amplified, may not show impairment in CA (39). These factors may limit the generalizability of resting CA assessments and lead to overstatement of the clinical relevance of the findings. Additionally, there are no universal standards for the parameter settings used in transfer function analysis or interpretation of subsequent results, which makes comparisons between studies problematic. Future work is needed to clarify differences in methods used to assess CA in hypoxemic states and evaluate if these changes are generalizable to clinical settings.

Most CA studies rely on transcranial Doppler measurements of flow velocity and assume that vessel diameter is unchanged, yet there is evidence to suggest that this assumption may be invalid at extreme altitudes (39, 40). Dilation of the MCA at

ALT1 may explain why MCAv did not follow the expected increase in CBF upon acute exposure to high altitude (30). We do not believe potential MCA dilation affected our interpretation because the phase shift - our primary criterion for assessing changes in CA – measures the relative timing of oscillations in ABP and CBFv and thus is largely independent of absolute flow. However, since small changes in diameter can have profound affects on flow (flow ~ radius^4), future studies must consider the use of continuous flow measurements, instead of velocity measurements, to accurately assess CA in hypoxia.

Finally, our measurements of CA were limited to the MCA and relied on pressure measurements taken in the radial artery. Since regional differences in cerebrovascular regulation have recently been reported (20, 28, 38), more specific measurements of regional pressure and flow are needed to fully characterize CA.

Conclusions

Our data demonstrate that the initial impairment of CA upon acute exposure to high altitude is invariant with acclimatization and re-exposure, suggesting that relaxation in the regulation of the cerebral pressure-flow relation is a characteristic response to hypoxia that is unaffected by the degree of acclimatization. Since changes in CA do not follow the progression and resolution of AMS, we question the clinical relevance of impaired CA at high altitude.

Acknowledgements

This paper is part of a series titled "AltitudeOmics" that together represent a group of studies that explored the basic mechanisms controlling human acclimatization to hypoxia and its subsequent retention. Many people and organizations invested enormous amounts of time and resources to make AltitudeOmics a success. Foremost, the study was made possible by the tireless support, generosity and tenacity of our research subjects. AltitudeOmics principal investigators were Colleen G. Julian, Andrew T. Lovering, Andrew W. Subudhi and Robert C. Roach. A complete list of other investigators on this multinational, collaborative effort involved in development, subject management and data collection, supporting industry partners, and people and organisations in Bolivia that made AltitudeOmics possible is available elsewhere (32).

Grants

The overall AltitudeOmics study was funded, in part, by grants from the U.S. Department of Defense (W81XWH-11-2-0040 TATRC to RR, and W81XWH-10-2-0114 to ATL); the Cardiopulmonary & Respiratory Physiology Laboratory, University of Oregon; the Altitude Research Center and the Charles S. Houston Endowed Professorship, Department of Emergency Medicine, School of Medicine, University of Colorado Denver.

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Figure Captions

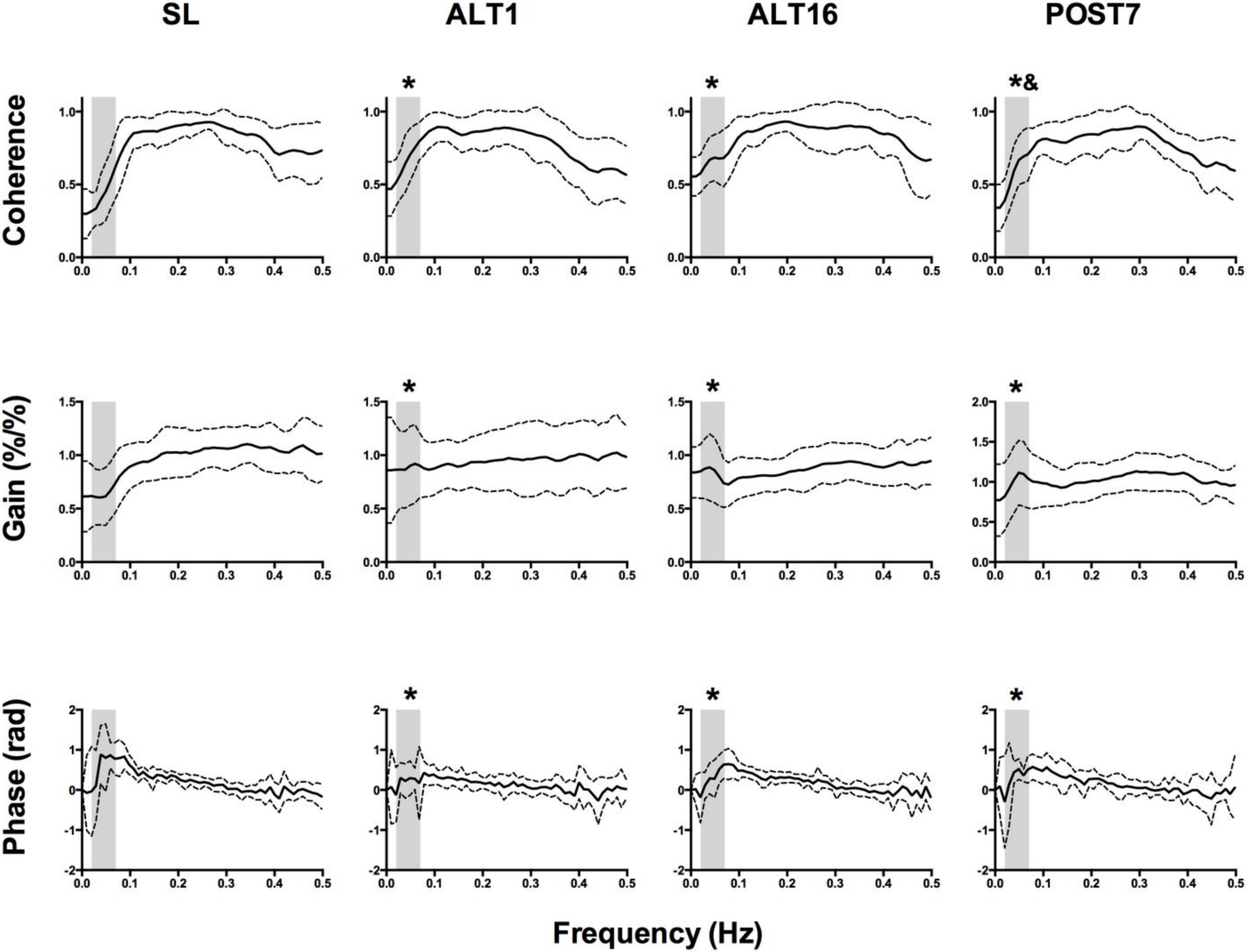
Figure 1. Arterial blood pressure to cerebral blood flow velocity transfer function metrics (mean ± SD from 0 to 0.5 Hz) at sea level (SL), upon arrival at 5,260 m (ALT1), after 16 days of acclimatization (ALT16), and upon re-exposure to 5,260 m after 7 days at low altitude (POST7). Similar impairments in cerebral autoregulation (increased coherence and gain and decreased phase shift) from SL were seen in the very low frequency (0.02 to 0.07 Hz – shaded area) at ALT1, ALT16, and POST7 ($P < 0.05$). * Different from SL. & Different from SL.

Figure 2. Scatter plots showing no relation ($P > 0.05$) between autoregulation indices (ARI), measured at sea level (SL, top) and as the change from SL to arrival at high altitude (ALT1, bottom), and AMS symptoms scores from the Lake Louise Questionnaire at ALT1.

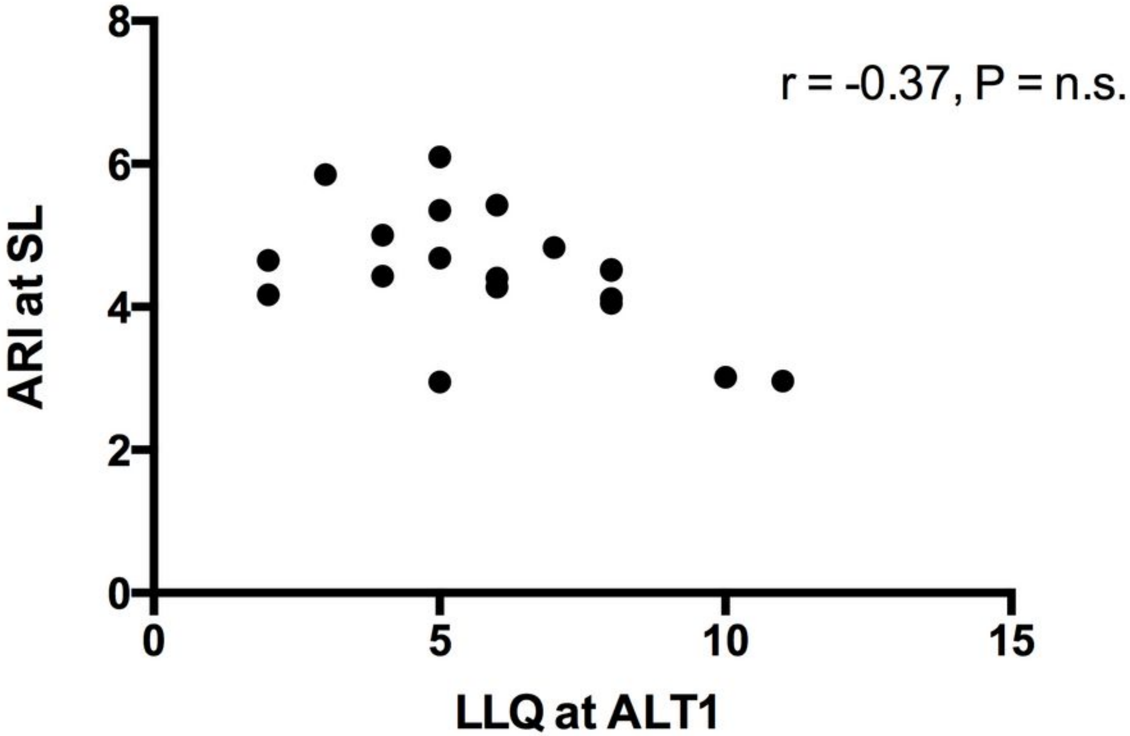
Table 1. Resting Data (n=14, mean ±SD)

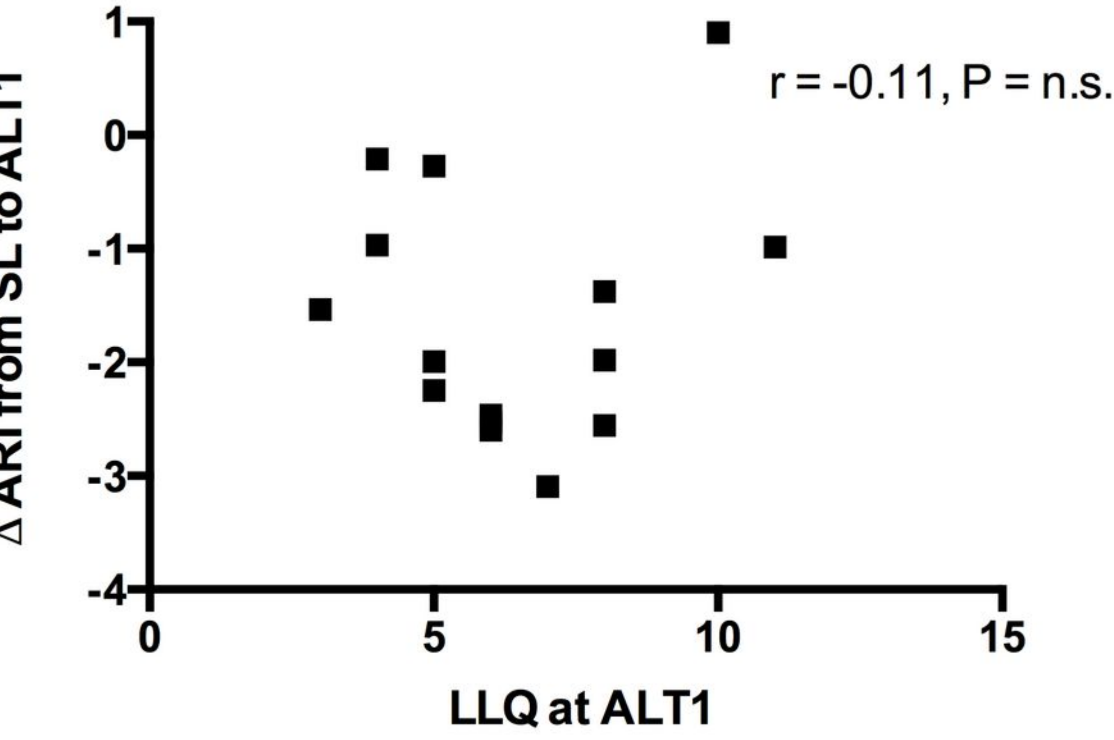
Variable		SL	ALT1	ALT16	POST7
PaO_2	mmHg	103 ± 5	36 ± 3*	45 ± 4*#	42 ± 4*#&
$PaCO_2$	mmHg	37 ± 4	28 ± 2*	21 ± 3*#	24 ± 3*#&
HR	bpm	73 ± 9	90 ± 18*	95 ± 12*	85 ± 15*&
ABP	mmHg	77 ± 6	76 ± 14	81 ± 10	76 ± 8
CBFv	cm/s	62 ± 9	63 ± 14	59 ± 7	57 ± 9
PSD ABP	$mmHg^2/Hz$	11 ± 13	9 ± 4	9 ± 5	6 ± 4
PSD CBFv	$(cm/s)^2/Hz$	13 ± 19	14 ± 16	10 ± 6	11 ± 8
Coherence		0.42 ± 0.12	0.64 ± 0.15*	0.70 ± 0.16*	0.55 ± 0.12*&
Gain	%/%	0.64 ± 0.24	0.88 ± 0.35*	0.85 ± 0.25*	0.97 ± 0.33*
Phase Shift	radians	0.48 ± 0.28	0.17 ± 0.21*	0.27 ± 0.09*	0.25 ± 0.19*
ARI		4.4 ± 1.0	2.8 ± 0.9*	2.8 ± 1.0*	3.3 ± 1.6*

* Different from SL
# Different from ALT1
& Different from ALT16



SL
ALT1
ALT16
POST7
Coherence
Gain (%/%)
Phase (rad)
*
*&
Frequency (Hz)


r = -0.37, P = n.s.
ARI at SL
LLQ at ALT1
8
6
4
2
0
0
5
10
15


r = -0.11, P = n.s.
Δ ARI from SL to ALT1
LLQ at ALT1
1
0
-1
-2
-3
-4
0
5
10
15