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

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The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
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.
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover</td>
<td>1</td>
</tr>
<tr>
<td>Report Documentation Page</td>
<td>2</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>3</td>
</tr>
<tr>
<td>Introduction</td>
<td>4</td>
</tr>
<tr>
<td>Body</td>
<td>4</td>
</tr>
<tr>
<td>Key Research Accomplishments</td>
<td>4</td>
</tr>
<tr>
<td>Reportable Outcomes</td>
<td>4</td>
</tr>
<tr>
<td>Conclusion</td>
<td>5</td>
</tr>
<tr>
<td>References</td>
<td>n/a</td>
</tr>
<tr>
<td>Appendices</td>
<td>6-129</td>
</tr>
</tbody>
</table>
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 abs 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

<table>
<thead>
<tr>
<th>#</th>
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<th>First/Last Authors</th>
<th>Status</th>
<th>PMID</th>
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<tbody>
<tr>
<td>1</td>
<td>Amann M, Goodall S, Twomey R, Subudhi AW, Lovering AT, Roach RC.</td>
<td>Amman/Roach</td>
<td>Published, JAPPL</td>
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<td>the development of fatigue during locomotor exercise in humans.</td>
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<td>2</td>
<td>Goodall S, Twomey R, Amann M, Ross EZ, Lovering AT, Romer LM,</td>
<td>Goodall/Roach</td>
<td>Accepted, Acta Scandinavica</td>
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<td>Subudhi AW, Roach RC. AltitudeOmics: Exercise-induced</td>
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<td>supraspinal fatigue is attenuated in healthy humans after</td>
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<td>3</td>
<td>Subudhi AW, Fan JL, Evero O, Bourdillon N, Kayser B, Julian CG,</td>
<td>Subudhi/Roach</td>
<td>Accepted, Exp Physiol</td>
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<td>4</td>
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<td>Fan/Roach</td>
<td>Accepted, JAPPL</td>
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<td>5</td>
<td>Subudhi AW, Fan JL, Evero O, Bourdillon N, Kayser B, Julian CG,</td>
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<td>6</td>
<td>Subudhi AW, Bucher J, Bourdillon N, Davis C, Elliott J, Eutermoster</td>
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<td>Accepted, PLOSOne</td>
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<td>7</td>
<td>AltitudeOmics: hemoglobin mass increases within 7 days of acclimatization to 5260m and is lost within 7 days of descent to 1525m</td>
<td>Ryan/Roach</td>
<td>Under revision</td>
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</tr>
<tr>
<td>8</td>
<td>AltitudeOmics: Detecting high altitude cognitive impairment with DANA, an Android-based neurocognitive assessment tool</td>
<td>Roach/Roach</td>
<td>Submitted, Neuroreport</td>
<td></td>
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</tr>
<tr>
<td>9</td>
<td>AltitudeOmics: Gene expression and epigenetics during ascent, acclimatization, and re-exposure to 5,260m</td>
<td>Julian/Roach</td>
<td>In prep</td>
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<td>10</td>
<td>AltitudeOmics: Effect of ascent, acclimatization, and re-exposure to 5,260m on pulmonary shunt</td>
<td>Elliot/Roach</td>
<td>In prep</td>
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<tr>
<td>11</td>
<td>AltitudeOmics: Effect of ascent, acclimatization, and re-exposure to 5,260m on muscle mitochondrial respiration</td>
<td>Chicco/Roach</td>
<td>In prep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>AltitudeOmics: Metabolomics during ascent, acclimatization, and re-exposure to 5,260m</td>
<td>Monte/Roach</td>
<td>In prep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>AltitudeOmics: Effect of ascent, acclimatization, and re-exposure to 5,260m on AMS and pulmonary shunt</td>
<td>Kern/Roach</td>
<td>In prep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>AltitudeOmics: Gene Expression and Acute Mountain Sickness</td>
<td>Kern/Roach</td>
<td>In prep</td>
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<td></td>
</tr>
<tr>
<td>15</td>
<td>AltitudeOmics: MicroRNA expression during ascent, acclimatization, and re-exposure to 5,260m</td>
<td>Kern/Roach</td>
<td>In prep</td>
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AltitudeOmics: on the consequences of high-altitude acclimatization for the development of fatigue during locomotor exercise in humans

Markus Amann,1 Stuart Goodall,2 Rosie Twomey,3 Andrew W. Subudhi,4,5 Andrew T. Lovering,6 and Robert C. Roach4

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

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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 (O2)-delivery sensitive [arterial O2 content (CtO2) × leg blood flow (Qt)]. Locomotor exercise in acute hypoxia (AH) is, compared with sea level (SL), associated with reduced CtO2 and exaggerated inspiratory muscle work (Winsp), which impair Qt, both of which exacerbate fatigue individually by compromising O2 delivery. Since chronic hypoxia (CH) normalizes CtO2 but exacerbates Winsp, 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 O2, 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 (∆Qtw,pot). Central fatigue was expressed as the exercise-induced percent decrease in voluntary muscle activation (∆VA). Resting CtO2 at SL and CH was similar, but CtO2 in AH was lower compared with SL and CH (17.3 ± 0.5, 19.3 ± 0.7, 20.3 ± 1.3 ml O2/dl, respectively). Winsp during exercise increased with acclimatization (SL: 387 ± 36, AH: 503 ± 53, CH: 608 ± 67 cmH2O·s-1·min-1; P < 0.01). Exercise at SL did not induce central or peripheral fatigue. ∆Qtw,pot was significant but similar in AH and CH (21 ± 2% and 19 ± 3%; P = 0.24). ∆VA was significant in both hypoxic conditions but smaller in CH vs. AH (4 ± 1% vs. 8 ± 2%; P < 0.05). In conclusion, acclimatization to altitude; respiratory muscle work; arterial O2 content; cerebral blood flow

Acute exposure to hypoxia (AH) has a substantial impact on the two determinants of leg muscle O2 delivery during strenuous locomotor exercise. First, despite a marked hyperventilatory response, arterial partial pressure of O2 [PO2 (P'O2)] and arterial hemoglobin saturation (SaO2) fall below sea level (SL) values and cause a significant reduction in CtO2. In addition, inspiratory muscle work (Winsp) is increased substantially at any given workload in hypoxia (2, 58), and these high levels of Winsp compromise, in a dose-dependent manner, Qt, during exercise (34). Each of these two determinants of leg muscle O2 delivery, namely CtO2 and Qt, 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 PO2, 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 O2 gradient, which combined, substantially improves arterial oxygenation during exercise by increasing P'O2 and SaO2 (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 CtO2 (8, 13). In contrast to this beneficial effect on O2 delivery, Qt, 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 O2 delivery depends on the degree to which the increase in CtO2 can counterbalance potential reductions in Qt. It has been documented previously that at a given absolute workload, locomotor muscle O2 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 O2 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 O2 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.

THE DEVELOPMENT OF LOCOMOTOR muscle fatigue during whole-body endurance exercise is highly sensitive to the delivery of oxygen [O2]; arterial O2 content (CtO2) × leg blood flow (Qt). Specifically, blunted O2 delivery exaggerates, and augmented O2 delivery attenuates the rate of development of locomotor muscle fatigue during exercise (1).
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 above 1,500 m and had not traveled to elevations above 1,500 m.

Experiments in AH were conducted at the same altitude, while breathing a gas mixture containing 10.5% O2. Balance nitrogen, and experiments in CH were conducted on the 14th day of acclimatization at 5,260 m (BP 408.9 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 ($W_{\text{peak}}$) was obtained from a maximal incremental exercise test (70, 100, 130, and 160 W for 3 min, each followed by 15 W/min increments thereafter) on a computer-controlled bicycle ergometer (Velotron, Dynaft; 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 $W_{\text{peak}}$ 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 $W_{\text{peak}}$ (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% $W_{\text{peak}}$ (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% O2) in AH.

Exercise Responses

Pulmonary ventilation ($V_{\text{E}}$) 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 O2 saturation ($S_{\text{a}}O_2$) 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. $C_{\text{O}_{2}}$ was estimated as 1.39 [Hb] × ($S_{\text{a}}O_2$/100). During all constant workload trials, esophageal pressure ($P_{\text{es}}$) was measured via a nasopharyngeal balloon (Cooper Surgical, Trumbull, CT), using standard procedures (7). To estimate $W_{\text{inact}}$, $P_{\text{es}}$ was integrated over the period of inspiratory flow, and the results were multiplied by respiratory frequency ($f_{\text{R}}$) 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 (O2Hb) 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 [O2Hb] 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 O2 delivery was calculated as the product of CBFv and $C_{\text{O}_{2}}$. 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 dilatation 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 $P_{\text{es}}$ 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 $V_{\text{T}}$. 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 (Teeda, 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 (Qtw,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/Qtw,pot)] × 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 (Qtw,pot; peak force − onset force), maximum rate of force development (MRFD), contraction time, maximum relaxation rate (MRR), and one-half relaxation time (RT0.5). 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 CVSs, acceptable degrees of reproducibility include: MVC, CV = 3.1%, r = 0.97; Qtw,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

C02 and Cerebral O2 Delivery

C02 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 O2/dl, respectively). Acclimatization to altitude significantly increased [Hb] and S02, resulting in similar C02 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 O2 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 Wimp work by 34 ± 8% above that at SL (P < 0.01) and dropped S02 by 36 ± 5% during the final minute of exercise. Following 14 days of acclimatization, Wimp was increased further by 23 ± 8% from AH, and S02, during the final minute of exercise, was 36 ± 5% higher in CH vs. AH. Breathing frequency and VE rose

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

<table>
<thead>
<tr>
<th></th>
<th>Sea Level</th>
<th>Acute Hypoxia</th>
<th>Chronic Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>152 ± 5</td>
<td>174 ± 4*</td>
<td>166 ± 4*</td>
</tr>
<tr>
<td>VTs, 1 min −1</td>
<td>64 ± 4</td>
<td>113 ± 8*</td>
<td>133 ± 10†</td>
</tr>
<tr>
<td>VTs, breaths min −1</td>
<td>32 ± 2</td>
<td>50 ± 3*</td>
<td>54 ± 3*</td>
</tr>
<tr>
<td>VTs, liter</td>
<td>2.0 ± 0.1</td>
<td>2.2 ± 0.2</td>
<td>2.6 ± 0.2*</td>
</tr>
<tr>
<td>VO2, 1 min −1</td>
<td>2.58 ± 0.19</td>
<td>2.44 ± 0.19*</td>
<td>2.39 ± 0.16*†</td>
</tr>
<tr>
<td>VO2, 1 min −1</td>
<td>2.51 ± 0.22</td>
<td>2.81 ± 0.21*</td>
<td>2.40 ± 0.15†</td>
</tr>
<tr>
<td>VO2/VO2, %</td>
<td>25 ± 1</td>
<td>50 ± 4*</td>
<td>56 ± 3*</td>
</tr>
<tr>
<td>VO2/VO2, %</td>
<td>26 ± 1</td>
<td>41 ± 2*</td>
<td>58 ± 3*</td>
</tr>
<tr>
<td>S02, %</td>
<td>94.1 ± 1.0</td>
<td>62.2 ± 1.8*</td>
<td>75.6 ± 1.2†</td>
</tr>
<tr>
<td>CBFv, cm/s</td>
<td>59.1 ± 4.8</td>
<td>74.2 ± 3.8*</td>
<td>73.2 ± 3.4*</td>
</tr>
<tr>
<td>Cerebral O2 delivery, a.u.</td>
<td>1.056 ± 0.62</td>
<td>895 ± 40†</td>
<td>1.289 ± 42†</td>
</tr>
<tr>
<td>fT/Ttot</td>
<td>0.35 ± 0.01</td>
<td>0.39 ± 0.01*</td>
<td>0.39 ± 0.01*</td>
</tr>
<tr>
<td>VTs, s</td>
<td>1.30 ± 0.08</td>
<td>0.74 ± 0.05*</td>
<td>0.70 ± 0.04*</td>
</tr>
<tr>
<td>Wimp, cmH2O · s −1 · min −1</td>
<td>387 ± 36</td>
<td>503 ± 53*</td>
<td>608 ± 67†</td>
</tr>
<tr>
<td>IC, liter</td>
<td>3.29 ± 0.22</td>
<td>3.13 ± 0.23</td>
<td>3.60 ± 0.23*</td>
</tr>
<tr>
<td>VO2/IC</td>
<td>0.60 ± 0.03</td>
<td>0.68 ± 0.02*</td>
<td>0.72 ± 0.02†</td>
</tr>
<tr>
<td>IRV, liter</td>
<td>1.30 ± 0.14</td>
<td>0.99 ± 0.13*</td>
<td>0.96 ± 0.05†</td>
</tr>
<tr>
<td>ERV, liter</td>
<td>1.98 ± 0.25</td>
<td>2.14 ± 0.29</td>
<td>1.67 ± 0.25†</td>
</tr>
<tr>
<td>EELV, %TLC</td>
<td>80.5 ± 1.6</td>
<td>85.4 ± 1.7*</td>
<td>85.2 ± 0.9*</td>
</tr>
<tr>
<td>Expiratory flow limitation, n</td>
<td>51.5 ± 1.8</td>
<td>53.8 ± 2.5</td>
<td>46.9 ± 2.1†</td>
</tr>
</tbody>
</table>

|                      |          |               |                |
|                      | out of 8 subjects | 0/8         | 2/8            |
| 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; VTs, minute ventilation; fT, breathing frequency; VTs, tidal volume; VO2, maximum oxygen (O2) uptake; VCO2, carbon dioxide production; S02, arterial O2 saturation; CBFv, cerebral blood flow velocity; Ti, duration of inspiration; Ttot, duration of entire breath; Tae, duration of expiration; Wimp, inspiratory muscle work; IC, inspiratory capacity; IRV, inspiratory reserve volume; ERV, expiratory reserve volume; EELV, 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 VT in two 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 ($P < 0.05$).

**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. HbO2 was unchanged from baseline to warm-up at SL ($P = 0.80$) but decreased significantly in AH and CH. Compared with baseline, HbO2 was unchanged during the final minute of exercise at SL ($P = 0.24$) but similarly increased in AH and CH (both $P < 0.01$). Hb was unchanged from baseline to warm-up in all three conditions. In contrast, compared with baseline, Hb 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 $C_eO_2$ and increased $W_{insp}$, resulted in a substantial degree of both peripheral and central fatigue. Two weeks of exposure to 5,260 m restored $C_eO_2$ 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).

![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.*](image-url)
expressed as means (10.6/H11006 pre-exercise baseline. All exercise trials were performed for the same duration
resting arterial oxygen (O2) content: 19.3
cle activation (VA; twitch force (Qtw,pot; M-wave amplitude 0.7
Voluntary muscle activation
MVC
quadriceps muscle function
Table 2. Effects of constant-load cycling exercise on
quadriceps muscle function
<table>
<thead>
<tr>
<th></th>
<th>Sea Level</th>
<th>Acute Hypoxia</th>
<th>Chronic Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qtw,pot</td>
<td>−3.1 ± 1.8*</td>
<td>−20.9 ± 2.4</td>
<td>−18.8 ± 3.4</td>
</tr>
<tr>
<td>MRFD</td>
<td>−4.1 ± 2.5*</td>
<td>−21.2 ± 4.2</td>
<td>−17.9 ± 3.5</td>
</tr>
<tr>
<td>MRR</td>
<td>2.7 ± 2.8*</td>
<td>−13.2 ± 3.1</td>
<td>−9.0 ± 2.2</td>
</tr>
<tr>
<td>RT0.5</td>
<td>1.0 ± 2.2*</td>
<td>9.2 ± 1.3</td>
<td>8.2 ± 1.4</td>
</tr>
<tr>
<td>MVC</td>
<td>−1.3 ± 1.2*</td>
<td>−22.3 ± 1.2</td>
<td>−8.9 ± 1.3†</td>
</tr>
<tr>
<td>Voluntary muscle activation</td>
<td>−0.1 ± 1.0*</td>
<td>−6.9 ± 1.1</td>
<td>−3.7 ± 1.2‡</td>
</tr>
<tr>
<td>M-wave amplitude</td>
<td>0.7 ± 2.7*</td>
<td>2.5 ± 2.0*</td>
<td>−7.8 ± 2.1</td>
</tr>
</tbody>
</table>

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. Qtw,pot, potentiated single twitch; MRFD, maximal rate of force development; MRR, maximal rate of relaxation; RT0.5, 1/2 relaxation time; MVC, maximal voluntary contraction force; M-wave, compound muscle action potential. Percent muscle activation is based on super-imposed 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, C3O2 was approximately one-third lower and Wm,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 C3O2 on muscle fatigability is mediated via the facilitating effects of the associated reduc-
tion in muscle O2 delivery on the intramuscular accumulation of metabolites known to cause peripheral fatigue, i.e., hydrogen ion and inorganic phosphate (37, 61). The Wm,Insp-induced exacerbation of peripheral fatigue results from the same intramuscular metabolic consequences associated with reductions in locomotor muscle O2 delivery. However, in the case of the Wm,Insp-related impairment in peripheral fatigue, the compromised O2 delivery is the consequence of a sympathetically mediated impact on QL, secondary to the activation of the respiratory muscle metaboreflex (34). Taken together, the combined effects of a significantly reduced C3O2 and a higher Wm,Insp has a profound impact on leg O2 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 O2 delivery during submaximal endurance exercise in AH and CH. Given the critical dependency of the development of peripheral fatigue on muscle O2 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 improve-
ment in leg muscle O2 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 (Qtw,pot; top) and voluntary muscle activation (VA; bottom) at sea level (SL; resting arterial oxygen (O2) content: 19.3 ± 0.7 ml O2/dl) and in AH (17.3 ± 0.5 ml O2/dl) and chronic hypoxia (CH; 20.3 ± 1.3 ml O2/dl).](J Appl Physiol • doi:10.1152/japplphysiol.00606.2013 • www.jappl.org)
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 O2 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 O2 delivery during exercise. Since \( CaO_2 \) was comparable at SL and CH (see RESULTS), the same exercise-induced increase in \( THb \) (Fig. 3) suggests a similar degree of O2 delivery in these conditions. Furthermore, the combination of a lower \( CaO_2 \) in AH vs. CH (and SL; see RESULTS) plus the similar increase in \( THb \) during exercise (Fig. 3) insinuates a lower locomotor muscle O2 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 O2 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 O2 in CH, i.e., a decreased capillary muscle O2 conductance (41). This impact might, despite a similar O2 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 \( CaO_2 \) and bulk O2 delivery, per se, might not depict key determinants of the exaggerated fatigability in hypoxia. Important here is the fact that despite the normalized \( CaO_2 \) and bulk O2 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 \( V_{E} \) 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 \( W_{insp} \)
observed in hypobaric CH is attributable to the exaggerated ventilatory response associated with altitude acclimatization.

The increase in minute \( V_t \) in the present study was mainly due to the increase in \( V_t \); \( Q_t \) 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 \( W_{\text{insp}} \) at the same workload in CH vs. AH resulted from the substantially higher \( V_t \) following acclimatization. Finally, this exaggerated \( W_{\text{insp}} \) likely aggravated the respiratory muscle metaboreflex and associated impact on leg vascular conductance (25) and presum-

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 fa-
tigue. 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 \( Q_{\text{tw,pot}} \)) in our experiments were similar in all three conditions. This suggests that neither severe AH nor CH impairs sarcolemma Na\(^+\)–K\(^+\)–ATPase ac-
tivity 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). How-
ever, 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-

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 mea-
sure 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 \( C_v O_2 \) and brain \( O_2 \) delivery were similar/higher in CH vs. SL, the still substantially lower \( P_v O_2 \) might have contributed to the greater degree of central fatigue during exercise in this condition. Indeed, a low \( P_v O_2 \) 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 \( C_v O_2 \) and \( P_v O_2 \) 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 ob-
served from AH to CH. We therefore propose that the significa-
tantly attenuated central fatigue during exercise in severe CH likely accounts, at least in part, for the improvement of endur-
ance performance associated with altitude acclimatization.

Mechanisms underlying the hypoxia-induced curtailment of central motor drive (i.e., increase in central fatigue) and end-

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above 70–75% \(\text{SpO}_2\). At more severe degrees of hypoxemia (<70% \(\text{SpO}_2\)), 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 \(\text{SpO}_2\) 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.

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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 Bisgard, Mr. Jonathan Elliot, Dr. Steve Laurie, Ms. Julia Kern, Ms. Kara 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. Ogiheno 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.

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DISCLOSURES

The authors declare no conflicts of interest.

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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±39 W, 10.1±1.4 min) on three occasions: 1) in normoxia (N, P O2, 147.1 mmHg); 2) in AH (F O2, 0.105; P O2, 73.8 mmHg); 3) after 14 days in CH (5,260 m; P O2, 75.7 mmHg). 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 O2 delivery index (MCA v×CaO2) 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 O2 delivery.

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

Glossary

Cao2, arterial O2 content; CSP, cortical silent period; ERT, estimated resting twitch; F O2, fraction of inspired O2; fr, 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 aO2, partial pressure of arterial O2; P O2, partial pressure of inspired O2; Q tw,pot, potentiated quadriceps twitch force; rMT, resting motor threshold; SIT, superimposed twitch; Spo2, arterial O2 saturation; TMS, transcranial magnetic stimulation; VC02, carbon dioxide output; Ve, minute ventilation; Vo2, oxygen uptake; Vt, 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 O₂ 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.

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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 O2 uptake [VO2max], 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 VO2max and peak workload (Wpeak); 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% Wpeak obtained in the preliminary trial: 1) to the limit of tolerance in acute normobaric hypoxia (AH: FiO2 = 0.105; Eugene, Oregon, barometric pressure [BP] = 750 ± 2 mmHg; P,O2 = 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,O2 = 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,O2 = 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 $M_{\text{max}}$. Supramaximal stimulation was ensured by increasing the final intensity by 30% (mean current $253 \pm 60$ 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 $\mu$V were evoked. Thereafter, stimulus intensity was reduced in 1% steps until an intensity was reached that elicited an MEP of at least 50 $\mu$V 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 $M_{\text{max}}$ 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% $W_{\text{peak}}$ (26 ± 8 W) before the workload was increased to 50% normoxic $W_{\text{peak}}$ (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: ($[Hb] \times 1.39 \times S_pO_2 / 100$). 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 O2cap, 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: \(1 - \frac{\text{SIT}}{\text{ERT}} \times 100\).

The peak-to-peak amplitude and area of evoked MEPs and \(M_{\text{max}}\) 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 \(M_{\text{max}}\) 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/\(M_{\text{max}}\), CV = 9.6%, ICC = 0.66; \(M_{\text{max}}\), 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; \(Q_{\text{tw},\text{pot}}\), 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).

$S_2O_2$ and MCAv data are shown in Figure 3. Acute exposure to hypoxia decreased $S_2O_2$ at rest (Δ7 ± 4%; P = 0.009) and during the final minute of exercise (Δ34 ± 10%; P < 0.001). Resting $S_2O_2$ 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 $S_2O_2$ were apparent in N (P > 0.702). Resting MCAv did not differ between conditions at baseline (pooled average, 54 ± 9 cm·s$^{-1}$; P = 0.544). MCAv did not increase from rest at any time point in N (P > 0.108). MCAv 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·L$^{-1}$ in N and 1.63 ± 0.31 g·L$^{-1}$ in CH (P = 0.005). Resting $P_2O_2$ was reduced in AH compared to N (39.1 ± 4.8 vs. 103.3 ± 8.7 mmHg, P < 0.001), but was still lower than N (P < 0.001). $C_4O_2$ was lower at rest in AH vs. N (19.8 ± 1.9 vs. 21.5 ± 2.9 ml·dl$^{-1}$; P = 0.013); during the final minute of exercise $C_4O_2$ in AH was 36 ± 8% lower than N (P < 0.001) and 22 ± 9% lower than in CH (P = 0.001). $C_4O_2$ was lower at rest in CH vs. N (19.4 ± 2.6 vs. 21.5 ± 2.9 ml·dl$^{-1}$; P < 0.001) and during the final minute of exercise (17.6 ± 2.9 vs. 21.2 ± 2.9 ml·dl$^{-1}$; P = 0.725). Consequently, cerebral O2 delivery index (MCAv × $C_4O_2$) 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 ($\dot{V}_\text{E}$) rose substantially over time in all conditions. $\dot{V}_\text{E}/\dot{V}_\text{CO}_2$ during the final minute of exercise in AH and CH was approximately twofold greater than in N ($P < 0.001$); $\dot{V}_\text{E}/\dot{V}_\text{CO}_2$ 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 $\dot{V}_\text{O}_2$ 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 ($P \geq 0.119$). $Q_{\text{tw,pot}}$ did not differ between conditions at baseline (AH, 107 ± 13 N; N, 105 ± 12 N; CH, 110 ± 16 N; $P = 0.752$). $Q_{\text{tw,pot}}$ 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 $M_{\text{max}}$ in CH displayed a twofold increase compared to AH and N ($P < 0.019$); however, the change in $M_{\text{max}}$ during MVC was not statistically significant ($P > 0.058$). Neither measure of $M_{\text{max}}$ changed pre- to post-exercise in any condition ($P \geq 0.610$). Pooled across conditions, pre-exercise ERT (mean $r^2 = 0.95$) was 70% of the pre-exercise $Q_{\text{tw,pot}}$ 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 ($P \geq 0.107$).

Corticomotor responses

$r\text{MT}$ 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/$M_{\text{max}}$ ratio increased for within contraction responses during CH (vs. AH 50 and 75% MVC; $P \leq 0.014$ 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 ($\Delta 11\%$, $P = 0.014$), but not in N ($\Delta 4\%$, $P = 0.298$) or CH ($\Delta 6\%$, $P = 0.174$); 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 (FIO2 = 0.14 – 0.10; resting SpO2 = 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 (FIO2 = 0.12; resting SpO2 = 75%). Moreover, the present study found a twofold increase in corticospinal excitability after 14 d acclimatisation to severe altitude (5,260 m, equivalent to FIO2 0.105; resting SpO2 = 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 (FIO2 = 0.12; resting SpO2 = 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 O2 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-excitible 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 O₂ therapy, demonstrating that the changes were O₂ 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\(_{a}\)O\(_2\) and further decrease in P\(_{a}\)CO\(_2\) 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\(_a\)O\(_2\). 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\(_a\)O\(_2\) 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\(_a\)O\(_2\) at rest and during exercise (Amman et al., 2006a). At altitude, despite significant hemoconcentration, C\(_a\)O\(_2\) actually falls from rest to submaximal/maximal exercise by 10-25% (Calbet et al., 2003). This would suggest that exercise C\(_a\)O\(_2\) calculations, based on a resting C\(_a\)O\(_2\) measure, might actually overestimate C\(_a\)O\(_2\) 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\(_{a}\)O\(_2\). In the present study the P\(_{a}\)O\(_2\) 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\(_{a}\)O\(_2\) and C\(_a\)O\(_2\) and the reduction in central fatigue that occurs after a period of acclimatisation warrants further investigation.
Technical Considerations

Exercising in a hypobatic 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 O₂ 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 FIO₂ (0.105) at sea level to obtain the same PIO₂ (~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 O₂ 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.

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Conflict of Interest
Nothing to declare

References


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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. $O_2$Hb, oxygenated haemoglobin; HHb, deoxygenated haemoglobin and THb, total haemoglobin.

**Figure 3.** Arterial oxygen saturation ($S_{O_2}$) (a), cerebral blood flow velocity (MCAv) (b) and middle cerebral artery $O_2$ delivery index (MCAv × $C_{O_2}$) 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.

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Acute Hypoxia</th>
<th>Chronic Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats min⁻¹)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Rest</td>
<td>81 ± 7†</td>
<td>90 ± 9</td>
<td>104 ± 16</td>
</tr>
<tr>
<td>Final Min</td>
<td>150 ± 16*</td>
<td>173 ± 14</td>
<td>167 ± 16</td>
</tr>
<tr>
<td>$\dot{V}_E$ (l min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>14.3 ± 2.4</td>
<td>20.0 ± 2.6</td>
<td>24.5 ± 5.4</td>
</tr>
<tr>
<td>Final Min</td>
<td>60.0 ± 9.6**</td>
<td>108.8 ± 24.7†</td>
<td>128.5 ± 30.0</td>
</tr>
<tr>
<td>$f_R$ (breaths min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>15.6 ± 3.6</td>
<td>17.5 ± 4.5</td>
<td>13.0 ± 3.4</td>
</tr>
<tr>
<td>Final Min</td>
<td>31.4 ± 4.9**</td>
<td>51.6 ± 8.7†</td>
<td>54.8 ± 9.9</td>
</tr>
<tr>
<td>$V_t$ (l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>1.07 ± 0.37</td>
<td>1.30 ± 0.34</td>
<td>1.47 ± 0.63</td>
</tr>
<tr>
<td>Final Min</td>
<td>2.00 ± 0.45</td>
<td>2.07 ± 0.43</td>
<td>2.41 ± 0.58</td>
</tr>
<tr>
<td>$\dot{V}_O_2$ (l min⁻¹)</td>
<td></td>
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</tr>
<tr>
<td>Rest</td>
<td>0.49 ± 0.10</td>
<td>0.45 ± 0.08</td>
<td>0.45 ± 0.12</td>
</tr>
<tr>
<td>Final Min</td>
<td>2.45 ± 0.51</td>
<td>2.34 ± 0.58</td>
<td>2.07 ± 0.50</td>
</tr>
<tr>
<td>$\dot{V}_CO_2$ (l min⁻¹)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rest</td>
<td>0.44 ± 0.09</td>
<td>0.55 ± 0.09</td>
<td>0.39 ± 0.08</td>
</tr>
<tr>
<td>Final Min</td>
<td>2.32 ± 0.51</td>
<td>2.69 ± 0.62†</td>
<td>1.94 ± 0.50</td>
</tr>
<tr>
<td>$\dot{V}_t$/$\dot{V}_O_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>30.7 ± 2.7**</td>
<td>47.4 ± 6.5†</td>
<td>55.9 ± 14.9</td>
</tr>
<tr>
<td>Final Min</td>
<td>25.2 ± 2.4**</td>
<td>51.2 ± 15.0†</td>
<td>62.9 ± 9.2</td>
</tr>
<tr>
<td>$\dot{V}_t$/$\dot{V}_CO_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>33.9 ± 2.7†</td>
<td>37.9 ± 6.5†</td>
<td>63.4 ± 6.8</td>
</tr>
<tr>
<td>Final Min</td>
<td>26.2 ± 2.6**</td>
<td>41.7 ± 6.9†</td>
<td>67.1 ± 9.1</td>
</tr>
<tr>
<td>RPE, dyspnoea</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rest</td>
<td>7.0 ± 0.0</td>
<td>7.3 ± 0.5</td>
<td>7.1 ± 0.4</td>
</tr>
<tr>
<td>Final Min</td>
<td>11.4 ± 2.4**</td>
<td>19.4 ± 0.8</td>
<td>19.1 ± 0.7</td>
</tr>
<tr>
<td>RPE, limb</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rest</td>
<td>7.1 ± 0.4</td>
<td>7.1 ± 0.4</td>
<td>7.0 ± 0.0</td>
</tr>
<tr>
<td>Final Min</td>
<td>12.3 ± 3.3*</td>
<td>19.9 ± 0.4</td>
<td>17.6 ± 1.7</td>
</tr>
</tbody>
</table>

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.

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

Values are means ± SD for 7 participants. $M_{\text{max}}$, 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) 

Baseline | Warm up | Exercise
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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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 (DO₂) 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 DO₂ was calculated as the product of arterial oxygen content (CaO₂) 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 CaO₂ maintained global cerebral DO₂ across acclimatization, although DO₂ to the posterior cerebral circulation was increased by ~25% at ALT1 (P=0.032). CONCLUSIONS: Cerebral DO₂ 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 DO₂ 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\textsubscript{2} supply for cerebral function, longitudinal studies of
cerebral DO\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2}.
A more recent MRI study with a larger sample size reported a tendency towards
elevation of cerebral DO\textsubscript{2} after subjects returned from 2 days at 3,800 m (Smith et
al., 2013), but no measurements of regional cerebral DO\textsubscript{2} were made. Based the
limited data to date, it is uncertain if global or regional cerebral DO\textsubscript{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 O₂cap, Mountain View, CA, USA) systems, calibrated with the same 3-L syringe and known concentrations of O₂ and CO₂ 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$_{\text{flow}}$ and VA$_{\text{flow}}$) was determined
using standard, validated ultrasound techniques (Hoskins, 2008), where:
\[
X_{\text{Flow}} = \pi \times \left(\frac{\text{diameter in cm}}{2}\right)^2 \times \text{time averaged peak velocity in cm/s} \times 60 \text{ s}. 
\]
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:
\[
gCBF = (\text{ICA}_{\text{flow}} + \text{VA}_{\text{flow}}) \times 2. 
\]
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:
\[
\text{CVRi} = \frac{\text{mean ABP}}{X_{\text{flow}}} 
\]

**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). CaO₂ (vol%) was calculated as:

\[ \text{CaO}_2 = 1.39 \times [\text{Hb}] + \text{PaO}_2 \times 0.003 \]

Regional and global cerebral DO₂ were calculated as the products of CaO₂ and ICAₙₚₒₙ₁, VAₙₚₒₙ₁, 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 DO₂ 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 $\geq$ 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\textsubscript{2} was observed (P=0.039, Figure 2). Observed changes in measures of regional and global CBF and DO\textsubscript{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\textsubscript{2} and 9.2 ± 4.1 mmHg increase in PaO\textsubscript{2} (ALT1 vs. ALT16; all P < 0.001). SaO\textsubscript{2} and [Hb] rose 6 ± 5\% and 1.8 ± 0.9 g/dL, respectively, improving CaO\textsubscript{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\textsubscript{flow}, VA\textsubscript{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\textsubscript{2} fell from ALT1 to ALT16 (ICA DO\textsubscript{2} P = 0.028, VA DO\textsubscript{2} P = 0.020, global DO\textsubscript{2} P = 0.011) as the reductions in CBF outweighed the increase in CaO\textsubscript{2} (Figure 1); however, neither global nor regional cerebral DO\textsubscript{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\textsubscript{2} was preserved across acclimatization through a changing balance between CBF and CaO\textsubscript{2}, but there was slight increase in relative DO\textsubscript{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 /
% SaO₂ and a CO₂ reactivity of 4% CBF / mmHg CO₂ 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 SaO₂ could be expected to reduce CBF by ~15% and the 5.5 mmHg decrease in PaCO₂ 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 CO₂ 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 O₂ and CO₂ are sufficient to maintain tight control over cerebral DO₂ in hypoxia. Consistent delivery of oxygen may help offset the decreased PO₂ 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 DO₂ through the posterior cerebral circulation upon arrival at high altitude (Table 2) that dissipated with acclimatization. The acute increase in DO₂ 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 DO2 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 DO2 we speculate that the increased VA DO2 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 DO2 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 DO2 (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 DO₂, 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\textsubscript{2} to yield DO\textsubscript{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\textsubscript{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\textsubscript{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 \textit{et al.}, 2012) and argued to support dilation of the MCA in hypoxia (Wilson \textit{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 \textit{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.

\textbf{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\textsubscript{2} and CO\textsubscript{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.

\textbf{Competing Interests}

The authors have no conflicts or competing interests to disclose.
Funding

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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).
References


Bert P (1943). *Barometric Pressure*. College Book Company, Columbus, Ohio.


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

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

* Different from SL
# Different from ALT1
Table 2. Cerebrovascular values [mean± SD (n)]

<table>
<thead>
<tr>
<th>Variable</th>
<th>SL</th>
<th>ALT1</th>
<th>ALT16</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA Dia</td>
<td>cm</td>
<td>0.51 ± 0.08 (21)</td>
<td>0.54 ± 0.07 (16)</td>
</tr>
<tr>
<td>ICA Vel</td>
<td>cm/s</td>
<td>29.8 ± 8.2 (21)</td>
<td>38.9 ± 8.1 (16)*</td>
</tr>
<tr>
<td>ICA Flow</td>
<td>ml/min</td>
<td>384 ± 197 (21)</td>
<td>556 ± 203 (16)*</td>
</tr>
<tr>
<td>ICA CVRi</td>
<td>mmHg/ml/min</td>
<td>0.25 ± 0.12 (21)</td>
<td>0.16 ± 0.09 (16)*</td>
</tr>
<tr>
<td>VA Dia</td>
<td>cm</td>
<td>0.36 ± 0.06 (20)</td>
<td>0.41 ± 0.06 (16)*</td>
</tr>
<tr>
<td>VA Vel</td>
<td>cm/s</td>
<td>21.4 ± 4.4 (20)</td>
<td>24.4 ± 6.4 (16)</td>
</tr>
<tr>
<td>VA Flow</td>
<td>ml/min</td>
<td>133 ± 47 (20)</td>
<td>206 ± 98 (16)*</td>
</tr>
<tr>
<td>VA CVRi</td>
<td>mmHg/ml/min</td>
<td>0.66 ± 0.24 (20)</td>
<td>0.46 ± 0.28 (16)*</td>
</tr>
<tr>
<td>gCBF</td>
<td>ml/min</td>
<td>1057 ± 413 (20)</td>
<td>1524 ± 456 (16)*</td>
</tr>
<tr>
<td>gCBF CVRi</td>
<td>mmHg/ml/min</td>
<td>0.09 ± 0.03 (20)</td>
<td>0.05 ± 0.02 (16)*</td>
</tr>
<tr>
<td>DO2 ICA</td>
<td>ml/min</td>
<td>75 ± 37 (21)</td>
<td>84 ± 32 (16)</td>
</tr>
<tr>
<td>DO2 VA</td>
<td>ml/min</td>
<td>26 ± 10 (20)</td>
<td>31 ± 16 (16)*</td>
</tr>
<tr>
<td>DO2 gCBF</td>
<td>ml/min</td>
<td>206 ± 79 (20)</td>
<td>230 ± 74 (16)</td>
</tr>
<tr>
<td>MCAv</td>
<td>cm/s</td>
<td>59.5 ± 10.3 (21)</td>
<td>61.1 ± 13.3 (17)</td>
</tr>
<tr>
<td>MCA CVRi</td>
<td>mmHg/cm/s</td>
<td>1.36 ± 0.25 (21)</td>
<td>1.28 ± 0.32 (17)</td>
</tr>
<tr>
<td>ICA %Q</td>
<td>%</td>
<td>5.4 ± 2.7 (21)</td>
<td>7.6 ± 2.7 (15)*</td>
</tr>
<tr>
<td>VA %Q</td>
<td>%</td>
<td>1.9 ± 0.8 (20)</td>
<td>2.6 ± 1.1 (15)*</td>
</tr>
<tr>
<td>gCBF %Q</td>
<td>%</td>
<td>15.0 ± 5.8 (20)</td>
<td>20.4 ± 6.2 (15)*</td>
</tr>
</tbody>
</table>

* 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 (DO₂) increases in the vertebral artery (VA), but not internal carotid artery (ICA) at ALT1. Regional DO₂ is reduced with acclimatization, but not below sea level (SL) values. * Different from SL. # Different from arrival at altitude (ALT1).
AltitudeOmics: Enhanced cerebrovascular reactivity and ventilatory response to CO₂ with high altitude acclimatisation and re-exposure

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Running head: Cerebral function at altitude

Key words: cerebral blood flow, cerebral CO₂ 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 CO₂. 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 (VE) to varied levels of CO₂ 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 VE responses to CO₂ 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 VE responses to CO₂ compared to ALT1 (by 89±70% and 48.3±32.0 L/min, respectively, P<0.001). The acclimatisation gained for VE responses to CO₂ 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 VE responses to CO₂ 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 CO₂. Our data further demonstrates that this enhanced ventilatory CO₂ 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 CO₂, termed cerebrovascular CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ reactivity during steady-state hypoxic hypercapnia following 5 days at 4,350 m. Thus, the effect of altitude acclimatisation on cerebrovascular CO₂ reactivity remains unclear.

In addition, it is unknown if and for how long changes in cerebrovascular CO₂ reactivity from acclimatisation persist after descent. Repetitive seven-month exposures to high altitude were reported to improve arterial O₂ saturation (SaO₂), 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 CO₂ has yet to be examined.

Changes in cerebrovascular CO₂ reactivity with high-altitude acclimatisation depend on the method of assessment. At sea level, the steady-state method results in higher cerebrovascular CO₂
reactivity (40-42) and lower ventilatory CO₂ sensitivity (6, 18, 23, 55) compared to the modified rebreathing test. These differences have been attributed to the presence of a PCO₂ 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 VE and subsequent underestimation of the ventilatory CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ reactivity tests. The cerebrovascular CO₂ reactivity tests consisted of: i) 10 min with end-tidal PCO₂ (PETCO₂) clamped at 40 mmHg; ii) 3 min voluntary hyperventilation to lower PETCO₂ to ~20 mmHg; iii) the modified rebreathing test (details below); and iv) 3 min with
PETCO₂ clamped at 50 mmHg. The entire cerebrovascular CO₂ reactivity protocol was carried out in background of hyperoxia (end-tidal PO₂ [PETO₂] > 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 PETCO₂ at 40 and 50 mmHg. Throughout the end-tidal PCO₂ clamping, we maintained PETO₂ at >250 mmHg by titrating 50% or 100% O₂ 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 CO₂ reactivities (14, 16, 34, 41). By using hyperoxia (PETO₂ > 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 CO₂ 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 PCO₂ and PO₂ 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 PETCO₂ at 20 mmHg at both sea level and 5,260 m (in background PETO₂ > 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 PETCO₂ reached 50 mmHg, PETO₂ 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 PETCO₂ of 20 mmHg and expressed as percentage change.

Respiratory variables: V̇E 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. PETO₂ and PETCO₂ were measured using fast responding gas analysers (O₂Cap 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 O₂ and CO₂ 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, PO₂ (PaO₂), PCO₂ (PaCO₂) (Rapidlab™ 248, Siemens Healthcare Diagnostics Inc., Henkestrasse, Germany), haemoglobin concentration ([Hb]) and O₂ saturation (SaO₂) (Radiometer OSM3, Radiometer Medical ApS, Copenhagen, Denmark). The blood gas values were analysed in triplicate and temperature corrected (26, 53).

Arterial bicarbonate concentration ([HCO₃⁻]) 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 PETCO₂ clamping at 50 mmHg at ALT16, the steady-state MCAv-CO₂, MAP-CO₂ and CVCi-CO₂ slopes were estimated from the difference in mean MCAv, MAP and CVCi at the end of 20 and 40 mmHg PETCO₂ clamp (20 sec averages), plotted against the change in PaCO₂ between these two conditions across all time points (SL, ALT1, ALT16, POST7 and POST21). The absolute value of \( \bar{V}E \) at clamp 40 mmHg was used as an estimate of steady-state \( \bar{V}E \) responsiveness to CO₂, since voluntary hyperventilation was necessary to reduce PETCO₂ 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$\text{2}$ 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$\text{2}$ 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$\text{2}$ slope during the modified rebreathing. For comparison, we plotted the MCAv-CO$\text{2}$ 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:

$$MCAv = a + (b/(1 + \exp(-(PETCO_2 - c)/d)))$$

Where MCAv is the dependent variable in cm/s, PETCO$\text{2}$ 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$\text{2}$ slope obtained from these two models ($R^2=0.71$). However, due to the range of PETCO$\text{2}$ 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$\text{2}$ 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-CO₂ slope, CVCi-CO₂ slopes and V̇E 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-CO₂ and V̇E-CO₂ 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 (α = 0.05, β =
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). V̇E 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 V̇E at 40 mmHg was retained by 38% at POST7 (P=0.004 vs. ALT1). Compared to ALT16, V̇E 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 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 $V_E$ 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 $V_E$ at 40 mmHg and the rebreathing $V_E$-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\textsubscript{2} reactivity using both the steady-state and modified rebreathing methods. We demonstrate that cerebrovascular CO\textsubscript{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\textsubscript{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\textsubscript{2} correlated with the changes in resting arterial [HCO\textsubscript{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\textsubscript{2} reactivity**

Our findings extend those from Fan et al., (16, 17) by demonstrating that the MCAv-CO\textsubscript{2} slope is elevated upon arrival at 5,260m and further elevated following 16 days of acclimatisation (Fig. 1A). Importantly, previous studies by Fan et al., (16, 17) assessed MCAv-CO\textsubscript{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\textsubscript{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\textsubscript{2} was not controlled, the MCAv-CO\textsubscript{2} slopes reported by Lucas et al., (30) reflect MCAv changes from hypoxic hypocapnia (room air breathing at 5,050 m; PETO\textsubscript{2} •48 mmHg & PETCO\textsubscript{2} 26-22 mmHg) to
hypercapnic hyperoxia (PETO₂ > 310 mmHg & PETCO₂ • 30 mmHg), and thus do not represent isolated reactivity to CO₂. Rupp et al., (49) recently found the MCAv response to steady-state hypoxic hypercapnia (PETO₂ = 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 PETO₂ (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 CO₂ 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 PaCO₂. In support of this notion, lowering CSF bicarbonate concentration elevates the cerebrovascular CO₂ 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 CO₂ 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 CO₂ with acclimatisation to high altitude and re-exposure (Fig. 1), which occurred in parallel to the changes in [HCO₃⁻] (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 CO₂ at high altitude remains to be further studied.

Interaction between cerebrovascular and ventilatory responsiveness to CO₂

Interaction between cerebrovascular CO₂ 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 CO₂ reactivity affect the stability of ventilatory response to CO₂ by modulating the degree of H⁺ washout at the level of the central chemoreceptor (38). Accordingly, a blunted cerebrovascular CO₂ reactivity would lead to less central H⁺ washout and subsequently greater central chemoreceptor activation. Conversely, an enhanced cerebrovascular CO₂ reactivity would result in lower central [H⁺] and therefore lower ventilatory CO₂ sensitivity. In agreement with previous altitude studies (16, 17), we observed concomitant increases cerebrovascular and ventilatory responsiveness to CO₂ (Fig. 1). These findings seem to contradict the modulating role of cerebrovascular CO₂ 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 SpO₂ 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 SpO₂ 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 CO₂ 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 CO₂ over acclimatisation was retained at POST7 (Fig. 1C), while
essentially none of the gain in MCAv-CO₂ 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 CO₂ is partially retained
after 7 days at low altitude, while it is reversed in the cerebrovascular response to CO₂. 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-CO₂ slope to be
consistently elevated by 60-80% across all time points (Fig. 1D), while the changes MAP-CO₂ slope
closely follows the changes in MCAv-CO₂ slopes (Fig. 1B), we speculate that the changes in MCAv-
CO₂ slopes at high altitude can be primarily accounted for by an enhanced sensitivity of the
cerebral vessels to CO₂, 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.

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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,
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organisations in Bolivia that made AltitudeOmics possible is available in the first paper in this
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M. Beasley for their invaluable assistance in the blood gas data collection for this study. We
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analysis program. We would like to thank Roberto Molinari for his assistance in the statistical
analysis of the data.


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

<table>
<thead>
<tr>
<th></th>
<th>SL (n=21)</th>
<th>ALT1 (n=17)</th>
<th>ALT16 (n=20)</th>
<th>POST7 (n=14)</th>
<th>POST21 (n=5)</th>
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<tr>
<td><strong>Steady-state</strong></td>
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<tr>
<td>MCAv-PaCO₂ slope (cm/s/mmHg)</td>
<td>1.19 ± 0.42</td>
<td>2.16 ± 1.05*</td>
<td>3.39 ± 0.89*†</td>
<td>2.68 ± 0.88§</td>
<td>2.06 ± 0.57§</td>
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<tr>
<td>CVCi-PaCO₂ slope (%/mmHg)</td>
<td>3.35 ± 1.21</td>
<td>5.87 ± 2.60*</td>
<td>5.75 ± 1.85*</td>
<td>5.89 ± 1.23*</td>
<td>5.41 ± 1.78*</td>
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<tr>
<td>MAP-PaCO₂ slope (L/min)</td>
<td>0.03 ± 0.24</td>
<td>0.28 ± 0.19*</td>
<td>1.06 ± 0.45†</td>
<td>0.56 ± 0.29§</td>
<td>0.32 ± 0.18§</td>
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<tr>
<td>V̇E at 40 mmHg (L/min)</td>
<td>19.15 ± 4.89</td>
<td>34.06 ± 12.23*</td>
<td>80.05 ± 32.32*†</td>
<td>49.03 ± 13.68*§†</td>
<td>43.25 ± 7.56*§</td>
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<tr>
<td><strong>Modified rebreathing</strong></td>
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<tr>
<td>MCAv-PETCO₂ slope (cm/s/mmHg)</td>
<td>1.34 ± 0.60</td>
<td>2.95 ± 1.11*</td>
<td>3.67 ± 0.87†</td>
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<tr>
<td>V̇E-CO₂ slope (L/min/mmHg)</td>
<td>1.90 ± 0.81</td>
<td>3.49 ± 1.51*</td>
<td>6.28 ± 3.56†</td>
<td>-</td>
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<tr>
<td>V̇E recruitment threshold (mmHg)</td>
<td>38.7 ± 3.4</td>
<td>33.7 ± 3.7*</td>
<td>29.2 ± 2.1†</td>
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* 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 3

(A) Rebreathing MCAv-CO₂ slope (cm/s/mmHg) vs. Steady-state MCAv-CO₂ slope (cm/s/mmHg)

- SL (black circles)
- ALT1 (red squares)
- ALT16 (blue triangles)

R = 0.860*

(B) Rebreathing VE-CO₂ slope (L/min/mmHg) vs. VE at 40 mmHg (L/min)

R = 0.904*
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 5,260 m 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, O₂ and CO₂) 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 PaCO₂ and PaO₂ 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 (α = 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, PaO2 and PaCO2 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 PaO₂ (2, 35) may have been masked if the opposing effects of PaCO₂ (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 hypoxic 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 \( (\text{flow} \sim \text{radius}^4) \), 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

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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)

<table>
<thead>
<tr>
<th>Variable</th>
<th>SL</th>
<th>ALT1</th>
<th>ALT16</th>
<th>POST7</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO₂</td>
<td>103 ± 5</td>
<td>36 ± 3*</td>
<td>45 ± 4*#</td>
<td>42 ± 4*#&amp;</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>37 ± 4</td>
<td>28 ± 2*</td>
<td>21 ± 3*#</td>
<td>24 ± 3*#&amp;</td>
</tr>
<tr>
<td>HR</td>
<td>73 ± 9</td>
<td>90 ± 18*</td>
<td>95 ± 12*</td>
<td>85 ± 15*&amp;</td>
</tr>
<tr>
<td>ABP</td>
<td>77 ± 6</td>
<td>76 ± 14</td>
<td>81 ± 10</td>
<td>76 ± 8</td>
</tr>
<tr>
<td>CBFv</td>
<td>62 ± 9</td>
<td>63 ± 14</td>
<td>59 ± 7</td>
<td>57 ± 9</td>
</tr>
<tr>
<td>PSD ABP</td>
<td>11 ± 13</td>
<td>9 ± 4</td>
<td>9 ± 5</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>PSD CBFv</td>
<td>13 ± 19</td>
<td>14 ± 16</td>
<td>10 ± 6</td>
<td>11 ± 8</td>
</tr>
<tr>
<td>Coherence</td>
<td>0.42 ± 0.12</td>
<td>0.64 ± 0.15*</td>
<td>0.70 ± 0.16*</td>
<td>0.55 ± 0.12*&amp;</td>
</tr>
<tr>
<td>Gain</td>
<td>0.64 ± 0.24</td>
<td>0.88 ± 0.35*</td>
<td>0.85 ± 0.25*</td>
<td>0.97 ± 0.33*</td>
</tr>
<tr>
<td>Phase Shift</td>
<td>0.48 ± 0.28</td>
<td>0.17 ± 0.21*</td>
<td>0.27 ± 0.09*</td>
<td>0.25 ± 0.19*</td>
</tr>
<tr>
<td>ARI</td>
<td>4.4 ± 1.0</td>
<td>2.8 ± 0.9*</td>
<td>2.8 ± 1.0*</td>
<td>3.3 ± 1.6*</td>
</tr>
</tbody>
</table>

* Different from SL
# Different from ALT1
& Different from ALT16
$r = -0.37, P = n.s.$

$\Delta ARI$ from SL to ALT1

$\begin{array}{c}
\text{LLQ at ALT1} \\
\text{ARI at SL}
\end{array}$

$r = -0.11, P = n.s.$