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

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Abstract

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

Glossary

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

Introduction

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

Transcranial magnetic stimulation (TMS) has been used to specify the site of fatigue within the central nervous system in acute severe hypoxia (Goodall *et al*., 2012, Goodall *et al*., 2010). When TMS is delivered over the motor cortex during a maximal voluntary contraction (MVC), it is possible to detect a twitch-like increment in force in the active muscle. That is, despite maximal effort, motor cortical output at the time of stimulation is insufficient to drive the motoneurons maximally. An increase in this increment in force after exercise provides evidence of a reduced cortical voluntary activation, indicative of supraspinal fatigue (Gandevia *et al*., 1996, Todd *et al*., 2003). Further, EMG recordings in response to cortical stimuli (motor evoked potential [MEP]) can be monitored to assess changes in excitability of the brain to muscle pathway. Descending volleys evoked from cortical stimulation depend on the stimulus intensity and excitability of corticospinal cells, whereas responses in the muscle depend on transmission through relevant excitatory and inhibitory interneurons and excitability of the motoneuron pool (Taylor and Gandevia, 2001). Hypoxia affects

neuronal function *in-vitro* (Nieber *et al*., 1999), however, acute hypoxia appears to have negligible effects on resting MEPs elicited by TMS (Goodall *et al*., 2010, Rupp *et al*., 2012, Szubski *et al*., 2006). A MEP evoked during muscular contraction is followed by an interval of EMG silence, the so-called cortical silent period (CSP). The initial phase of the CSP has been attributed to inhibitory spinal mechanisms (Inghilleri *et al*., 1993), whereas the later period (>100 ms) represents increased cortical inhibition (Chen *et al*., 1999, Inghilleri *et al*., 1993, Taylor and Gandevia, 2001). Szubski *et al*. (2006) found a shorter CSP in acute hypoxia, suggestive of a reduced corticospinal inhibition during the exercise.

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

Methods

Ethical Approval

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

Participants

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

Experimental design

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

participants continued to breathe the hypoxic gas. Cerebrovascular, cardiorespiratory and perceptual responses, as well as EMG activity of the vastus lateralis (VL), were assessed throughout each trial.

Force and EMG recordings

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

Neuromuscular function

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

Femoral nerve stimulation

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

plateaus occurred in twitch amplitude and M_{max}. Supramaximal stimulation was ensured by increasing the final intensity by 30% (mean current 253 ± 60 mA).

Transcranial magnetic stimulation

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

Constant-load exercise

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

Tissue oxygenation and cerebrovascular responses

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

Cardiorespiratory and perceptual responses

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

Data analysis

Cortical voluntary activation was assessed by measuring the force responses to motor-cortex stimulation during submaximal and maximal contractions. Corticospinal excitability increases during

voluntary contraction (Rothwell *et al.*, 1991); thus, we estimated the amplitude of the resting twitch evoked by TMS (ERT; Goodall *et al.*, 2009, Sidhu *et al.*, 2009a). Cortical voluntary activation (%) was subsequently quantified using the equation: (1 – [SIT / ERT] × 100).

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

Reliability coefficients

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

Statistical analysis

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

Results

Exercise responses

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

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

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

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

Pre- and post-exercise responses

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

Neuromuscular responses

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

Corticomotor responses

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

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

Discussion

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

Supraspinal Fatigue

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

Corticomotor responses

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

TMS over the motor cortex preferentially activates corticospinal neurons trans-synaptically through excitatory interneurons and corticocortical axons (Di Lazzaro *et al*., 1998). The response to TMS critically depends on membrane excitability of motor cortical neurons and ion-channel function (Boroojerdi *et al*., 2001, Rothwell *et al*., 1991). *In vitro* investigations using isolated cerebral neurons from rats demonstrate that ion-channel function is affected by O_2 availability and that neuronal hyper-excitability is the consequence of chronic hypoxia (Donnelly *et al*., 1992). A heightened neural response is necessary to maintain membrane integrity and ionic homeostasis that occur from a period of insufficient metabolic activity (Nieber *et al*., 1999). Thus, the twofold increase in MEP observed in the present study might be due to facilitated cortical neurons acting to restore the loss of neuronal activity associated with a prolonged exposure to altitude. Additionally, an increased level of muscle sympathetic nerve activity (peroneal microneurography) has been reported during a prolonged stay at the same altitude as in the present study (Hansen and Sander, 2003). That study showed a significant increase in muscle sympathetic nerve activity just 3 days after exposure to high altitude, suggesting that the prolonged stay induced a striking and long-lasting sympathetic over-activity. More recently, Buharin *et al*. (2013) found that a transient increase in sympathetic nerve activity (induced via lower body negative pressure) enhances corticospinal excitability as identified using TMS. The mechanism responsible for the increase in corticospinal excitability was postulated

to be due to an elevated concentration of noradrenaline, a monoamine that is known to increase exponentially during sustained periods at altitudes exceeding 4,000 m (Cunningham *et al*., 1965, Mazzeo *et al*., 1994). Thus, the increased corticospinal excitability observed following 2 weeks of acclimatisation in the present study might be attributable, at least in part, to a heightened sympathetic nerve activity and associated increases in corticospinal excitability as well as hyper-excitable cerebral neurons. The increased corticospinal excitability in this investigation occurred in line with no symptoms of mountain sickness, a finding that opposes that of Miscio *et al*. (2009). Miscio *et al*. (2009) found that exposure to high altitude changes cortical excitability by affecting both inhibitory and excitatory circuits and that this is reflected in acute mountain sickness symptoms. This conclusion was based on a group of participants who resided at 4,554 m for only 3-5 days, a time frame in which acute mountain sickness is said to be most prominent (Hackett and Roach, 2001) and much shorter than the present study.

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

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

Hematological and cerebrovascular responses

Upon initial exposure to high altitude, acute hypoxia dilates cerebral arterioles thereby overriding the vasoconstrictive effect of hyperventilation-associated hypocapnia (Iwasaki *et al*., 2011). During a prolonged stay at altitude, hypocapnia further develops and arterial hypoxaemia is ameliorated, as

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

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

Technical Considerations

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

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

Conclusion

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

Author Contributions

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

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Figure Legends

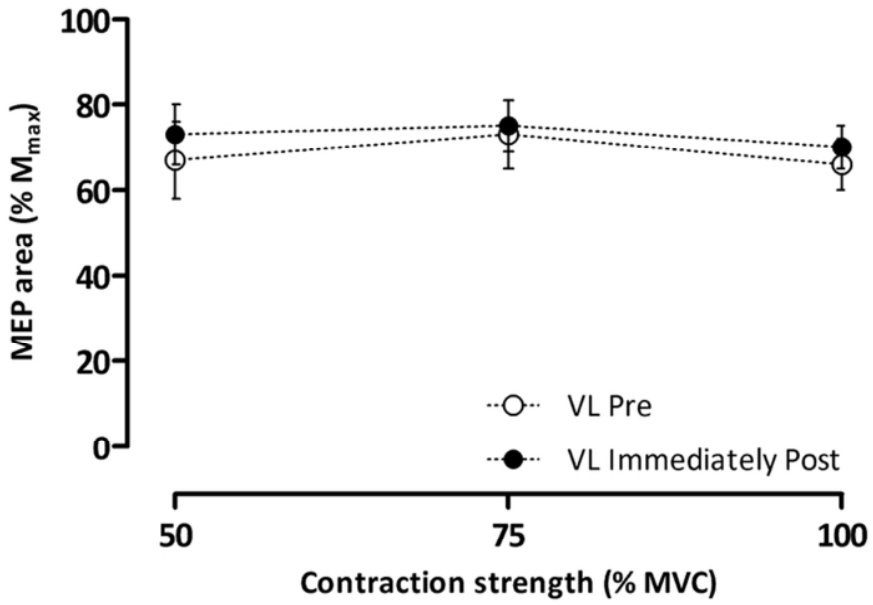
Figure 1. Mean area of motor evoked potentials (MEP) recorded from the vastus lateralis (VL) in response to stimulation over the motor cortex during varying contraction intensities pre- (○) and post-exercise (●) (mean for all conditions). The TMS responses were compared to the area of the maximal M-wave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

Figure 2. Cerebral oxygenation at resting baseline, during the final 30 s of a 3 min warm up (28 W) and during the final 30 s of constant-load exercise (131 W) in normoxia (N; panel a), acute hypoxia (AH; panel b) and chronic hypoxia (CH; panel c). Data are means ± SE for 7 participants. † $P < 0.05$ vs. respective baseline; ‡ $P < 0.05$ vs. respective warm up; * $P < 0.05$ vs. AH; # $P < 0.05$ vs. CH. Resting baseline in AH denotes the value after 10 min wash in of the hypoxic gas. O_2Hb, oxygenated haemoglobin; HHb, deoxygenated haemoglobin and THb, total haemoglobin.

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

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

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



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 (Mmax) evoked by peripheral stimulation of the femoral nerve. Data are means ± SE for 7 participants.
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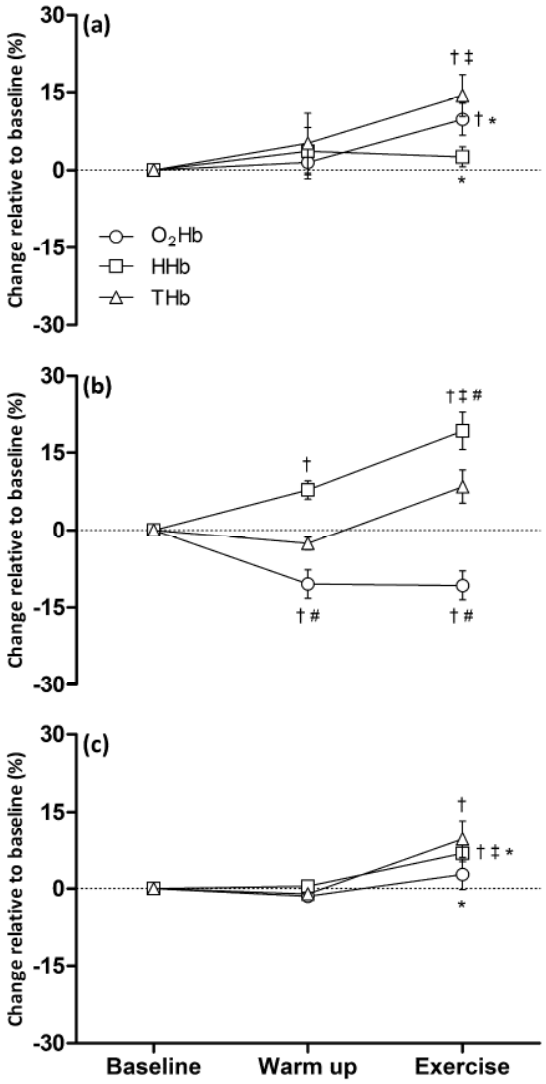

Cerebral oxygenation at resting baseline, during the final 30 s of a 3 min warm up (28 W) and during the final 30 s of constant-load exercise (131 W) in normoxia (N; panel a), acute hypoxia (AH; panel b) and chronic hypoxia (CH; panel c). Data are means ± SE for 7 participants. † $P < 0.05$ vs. respective 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. O2Hb, oxygenated haemoglobin; HHb, deoxygenated haemoglobin and THb, total haemoglobin.
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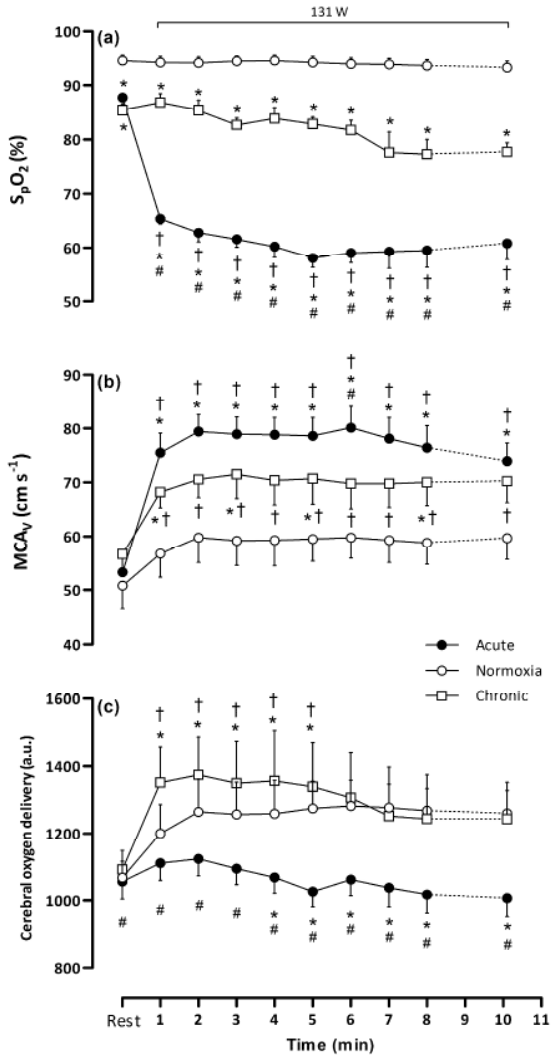
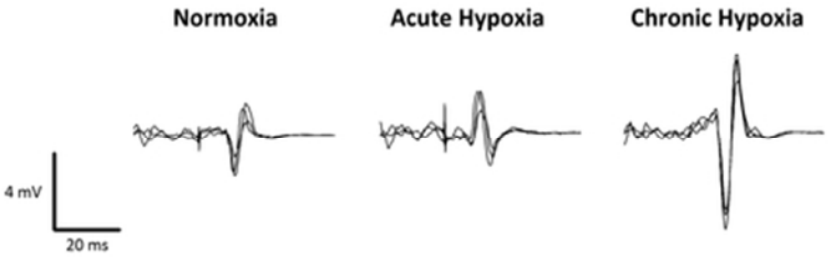
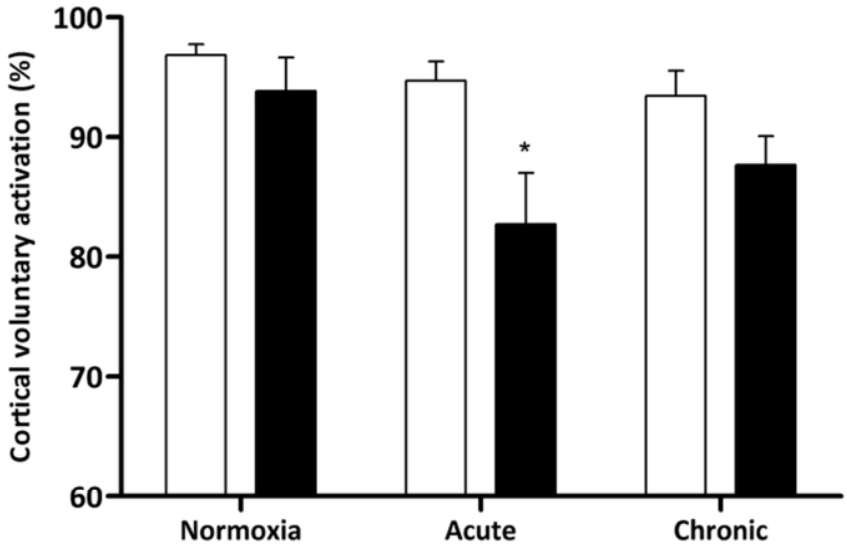


228x410mm (300 x 300 DPI)



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.
40x14mm (300 x 300 DPI)



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.
66x42mm (300 x 300 DPI)