Exercise-Induced Fatigue in Severe Hypoxia Following an Intermittent Hypoxic Protocol

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ABSTRACT

Purpose: Exercise-induced central fatigue is alleviated following acclimatisation to high altitude. The adaptations underpinning this effect may also be induced with brief, repeated exposures to severe hypoxia. The purpose of this study was to determine whether (i) exercise tolerance in severe hypoxia would be improved following an intermittent hypoxic (IH) protocol and (ii) exercise-induced central fatigue would be alleviated following an IH protocol. **Methods:** Nineteen recreationally-active males were randomised into two groups who completed ten 2-h exposures in severe hypoxia (IH: P_{iO_2} 82 mmHg; $n=11$) or normoxia (control; $n=8$). Seven sessions involved cycling for 30 min at 25% peak power ($\dot{W}_{peak}$) in IH, and at a matched heart rate in normoxia. Participants performed baseline constant-power cycling to task failure in severe hypoxia (TTF_{-Pre}). After the intervention, the cycling trial was repeated (TTF_{-Post}). Pre- and post-exercise, responses to transcranial magnetic stimulation and supramaximal femoral nerve stimulation were obtained to assess central and peripheral contributions to neuromuscular fatigue. **Results:** From pre- to post-exercise in TTF_{-Pre}, maximal voluntary force (MVC), cortical voluntary activation (VA_{TMS}) and potentiated twitch force ($Q_{tw,pot}$) decreased in both groups (all $p < 0.05$). Following IH, TTF_{-Post} was improved (535 ± 213 s vs. 713 ± 271 s, $p < 0.05$) and an additional isotime trial was performed. After the IH intervention only, the reduction in MVC and VA_{TMS} was attenuated at isotime ($p < 0.05$). No differences were observed in the control group. **Conclusion:** Whole-body exercise tolerance in severe hypoxia was prolonged following a protocol of IH. This may be related to an alleviation of the central contribution to neuromuscular fatigue.

Keywords: NEUROMUSCULAR; CYCLING; ALTITUDE; ACCLIMATIZATION;
HEMOGLOBIN MASS

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INTRODUCTION

Whole-body exercise tolerance in severe acute hypoxia (AH) is markedly impaired (4,14). The reduction in exercise tolerance in AH is not only a concern for mountaineers, but also military forces, where tactical necessity can result in rapid ascent of service personnel to high altitude and result in debilitating reductions in physical operational capabilities (28). At task failure following constant-power cycling in AH, neuromuscular fatigue is evident as a reduction in the ability to produce maximal isometric force, with a clear central contribution (4,17,19). Studies utilizing transcranial magnetic stimulation (TMS) before and after a fatiguing motor task in AH have shown that at least some of the resulting loss of force originates at or upstream of the motor cortex (17,19,36). This decrease in cortical voluntary activation (VA_{TMS}) occurs alongside pronounced cerebral deoxygenation and as such, exercise in AH is considered to be limited primarily by the hypoxic central nervous system (CNS) (11,47). Further evidence for this is provided from studies using an increase in the partial pressure of inspired O_2 (P_{iO_2}) at volitional exhaustion, where the capacity to resume whole-body exercise occurs too rapidly to be due to a reversal of metabolic disturbance in the locomotor muscles (e.g. (4)).

Initial evidence suggests that the mechanisms of exercise-induced fatigue in AH can be modulated by the physiological adaptations associated with acclimatization (3,19). A 14-day exposure to high altitude (P_{iO_2} 76 mmHg) alleviated exercise-induced central fatigue and this occurred alongside improvement in indices of systemic and cerebral O_2 availability (3,19). However, chronic exposure to hypoxia involves substantial logistical demand, immunological consequences (27), and risk of acute mountain sickness (AMS) (15). As such, intermittent

hypoxia (repeated exposures to sustained hypoxia lasting minutes to hours) via a decrease in P_{iO_2} , has been investigated as a means of promoting physiological adaptations without a prolonged stay at high altitude (or confinement to a hypoxic chamber) (28).

Following acclimatisation, a number of mechanisms may be responsible for an improved exercise tolerance in severe hypoxia (41). Hemoglobin concentration [Hb] is higher due to a reduction in plasma volume, and O_2 carrying capacity is improved via erythropoiesis (measured as an increase in total hemoglobin mass (37); Hbmass). Furthermore, hemoglobin saturation (S_pO_2) is increased during hypoxic exercise (3). These mechanisms contribute to an increase in arterial O_2 content (C_aO_2) during constant-power cycling in chronic hypoxia in comparison to AH (3). However, it is not necessarily the systemic improvement in C_aO_2 that results in an alleviation of central fatigue in chronic hypoxia, but may be the resulting improvement in cerebral O_2 delivery ($C\dot{D}O_2$) (19). $C\dot{D}O_2$ may be improved in the face of an unchanged cerebral blood flow (CBF), where CBF is subject to the opposing influences of hypocapnia-induced cerebral vasoconstriction (via hyperventilation) and hypoxia-induced cerebral vasodilation (1).

At least some of the adaptations that compensate for a reduced P_{iO_2} in chronic hypoxia may be achievable with an IH protocol. The principal beneficial response to IH that mimics acclimatization is considered to be an increase in C_aO_2 via early respiratory changes related to an increase in hypoxic chemosensitivity (7,10,28). In contrast, the evidence is largely unresponsive of an increase in [Hb] or Hbmass with IH protocols (26), likely due to an insufficient total duration of hypoxic exposure (32). However, alterations are more rapid in severe hypoxia (37), and may be possible with an IH protocol involving exercise training (34).

Few studies have investigated exercise tolerance in severe hypoxia following IH protocols conducted at the same P_{iO_2} , and some have shown improvements in whole-body exercise tolerance in the severe-intensity domain (e.g. (5,7)). However, neither of these examples included a control group. To our knowledge, no study has investigated central and peripheral fatigue following an IH intervention. Therefore, the aims of this study were to determine whether (i) exercise tolerance in severe hypoxia could be improved following an IH protocol in comparison to a control protocol in normoxia and (ii) exercise-induced central fatigue would be alleviated following an IH protocol. It was hypothesised that (i) an IH protocol would result in an improvement in exercise tolerance in severe hypoxia and (ii) the central contribution to neuromuscular fatigue would be alleviated following an IH protocol.

METHODS

Participants

Twenty-one participants were fully informed of the procedures and risks involved and provided written consent to participate. All participants were male, non-smokers, free from contraindications to experimental procedures including any history of cardiorespiratory or neurological disease, lowlanders, and had not visited altitudes ≥ 1000 m in the 3 months preceding the study. Participants were regularly physically active at a recreational level and were instructed to refrain from strenuous training for the duration of the experimental protocol. Participants were matched for normoxic peak O_2 uptake ($\dot{V}O_{2peak}$) and randomly assigned (GraphPad Software Inc, USA) to one of two treatment groups: IH or control. Nineteen participants completed all trials (IH: $n = 11$, $\dot{V}O_{2peak}$ 3.32 ± 0.42 L $\cdot$ min $^{-1}$, age 23 ± 2 years, height 180 ± 6 cm, body mass 76.4 ± 13.7 kg; and control: $n = 8$, 3.48 ± 0.36 L $\cdot$ min $^{-1}$, 22 ± 4 years, 180

± 6 cm, 83.0 ± 5.5 kg). The study was approved by the university research ethics committee and was performed according to the Declaration of Helsinki.

Experimental Design

For each participant, all testing was performed at the same time of day ± 1 h. Over two familiarization visits, participants were accustomed to (i) the neuromuscular assessment and cycle ergometer, and (ii), the optimised carbon monoxide rebreathing (oCO_r) method (Figure 1A). On a third visit, $\dot{V}O_{2\text{peak}}$ was obtained from a normoxic incremental cycling test ($5 \text{ W} \cdot 12 \text{ s}^{-1}$ from 80 W, preceded by a 3-min prior exercise at 50 W). Peak power ($\dot{W}_{\text{peak}}$) was derived as the highest power averaged over 30 s (IH: 309 ± 23 ; control: 313 ± 20 W). All subsequent cycling trials and both interventions were performed inside a large, purpose-built chamber (The Altitude Centre, UK; ambient temperature $19 \pm 1^\circ\text{C}$, relative humidity $40 \pm 2\%$, barometric pressure 760 ± 2 mmHg). Normobaric, poikilocapnic hypoxia was delivered and maintained at a $P_{\text{I}O_2}$ of 82 ± 1 mm Hg via nitrogen enrichment (equivalent to ~ 4700 m above sea level).

All participants performed a constant-power cycling trial to task failure (TTF) before the intervention (≥ 48 h after the incremental exercise test) and after the intervention (48 h after the final exposure). The pre-intervention TTF is referred to as TTF_{Pre}. Following the intervention, there was no significant change in the performance of the control group (see Results section). As such, the post-intervention TTF was treated as an 'isotime' trial (ISO). In the IH group, there was a significant increase in TTF post-intervention (TTF_{Post}). The IH group performed an additional isotime trial (ISO = TTF_{Pre}) 48 h after TTF_{Post}. Total Hb mass was measured before the intervention and 72 h after the final exposure.

The Intervention

Over 14 ± 2 days, participants in the IH group performed 10 hypoxic exposures of 2-h duration. The control group completed an identical protocol in normoxia (P_{iO_2} 149 ± 1 mmHg). During exposure 1, 5 and 10, participants remained seated for 2 h. During the remaining sessions, participants undergoing IH were seated for 90 min and cycled for 30 min at 25% $\dot{W}_{peak}$. During the first exercise bout in the control group, the power was adjusted to produce the same heart rate (HR) as for the IH group ($131 \text{ b} \cdot \text{min}^{-1}$). This power was fixed for the remaining exercise bouts ($38 \pm 2\% \dot{W}_{peak}$). Arterial O_2 saturation was estimated using a pulse oximeter (S_pO_2) with a fingertip sensor placed on the participant's right index finger (PalmSAT 2500 and 8000AA, Nonin Medical Inc, Minnesota, USA). HR (Polar Electro, Tampere, Finland) and S_pO_2 were recorded at 10-min intervals at rest, and 5-min intervals during exercise. Symptoms of AMS were assessed at 10-min intervals using the Lake Louise Questionnaire (LLQ) (33), with the sleep questions removed.

During the intervention, participants were naïve to the aims of the study and blinded to the O_2 levels inside the chamber, their HR, S_pO_2 and power during cycling. To assess the blinding procedures after completion of the final visit, participants were asked to complete a brief questionnaire to indicate whether (i) their exposures were in 'severe hypoxia (more than 4000 m above sea level)' or 'normoxia (sea level)', and (ii) if they were 'certain', 'fairly sure' or 'uncertain' about their answer. In response to (i), 53% of participants answered correctly and in response to (ii), 74% indicated that they were 'uncertain' about their answer.

Total Hemoglobin Mass

The oCO₂ was used to derive Hbmass, blood volume and plasma volume (38). A detailed description of the equipment and specific methods used has previously been described (45). Test-retest reliability was evaluated prior to the study with eight recreationally-active males (Hbmass: CV 3.9%, ICC 0.94, TEM 36 g; plasma volume: CV 3.3%, ICC 0.92, TEM 43 mL; blood volume: CV 3.1%, ICC 0.94, TEM 66 mL).

Constant-Power Cycling Trials

Upon arrival to the laboratory, participants provided a mid-stream urine specimen. Euhydration was accepted as a urine specific gravity of $< 1.020 \text{ g.mL}^{-1}$ and osmolality $< 700 \text{ mOsmols.kgH}_2\text{O}^{-1}$. Following 3 min at 50 W, power output was increased to 60% $\dot{W}_{\text{peak}}$ (IH: $187 \pm 14 \text{ W}$; control: $188 \pm 12 \text{ W}$). Trials were performed on an electromagnetically-braked cycle ergometer (SRM High Performance Ergometer; Schroeder Rad Meßtechnik, Jülich, Germany) with body position and self-selected cadence determined during familiarization, and replicated for the duration of the study. Cadence ($87 \pm 4 \text{ rev.min}^{-1}$) was the only real-time feedback participants received and verbal instructions were given should participants drift by $\geq 4 \text{ rev.min}^{-1}$ for $\geq 5 \text{ s}$. The nature and frequency of verbal encouragement was replicated across trials. Task failure was defined as a drop to $\leq 70\%$ of self-selected cadence for $> 5 \text{ s}$, despite strong verbal encouragement.

Within-Exercise Measures

Transcranial Doppler sonography was used to measure blood flow velocity in the left middle cerebral artery (MCA_v). The same experimenter isolated the motor cortex segment of the MCA

over the left temporal window using a 2 MHz probe on each visit. Signal quality was optimised and the probe was fixed in position within an adjustable headpiece. Continuous traces of the maximal velocity envelope were recorded and processed offline for determination of beat-by-beat mean velocity. Pulmonary ventilation and gas exchange were measured using a breath-by-breath system (Metalyzer 3B, Cortex Biophysik, Leipzig, Germany). Following orientation to the scales, RPE and breathlessness were obtained using the Borg RPE scale and the modified Borg CR10 Scale, respectively. RPE, breathlessness, HR, S_pO_2 were recorded at 1-min intervals. Resting [Hb] in combination with S_pO_2 was used to estimate C_aO_2 at rest and throughout exercise in all conditions using the equation $C_aO_2 = [Hb] \times 1.39 \times (S_pO_2 / 100)$. Subsequently, an index of $\dot{C}DO_2$ was calculated as the product of MCA_v and estimated C_aO_2 . The estimation of $\dot{C}DO_2$ in this study is based on the assumption that MCA_v is a valid surrogate of MCA blood flow. There are uncertainties about the constancy of MCA diameter which preclude this assumption (see (1)), and as such, the data should be interpreted with due caution.

Neuromuscular Assessment

Neuromuscular data were captured using a data acquisition system and analysed offline using custom-made macroinstructions (PowerLab 26T and LabChart Vv7, ADInstruments Ltd, Oxford, UK). Prior to the neuromuscular assessment, optimal coil position and resting motor threshold were determined (Figure 1B, described below). The neuromuscular assessment (Figure 1C) was completed in 205 s pre- and post-exercise and was performed within the chamber. The first maximal voluntary contraction (MVC) was performed ≤ 40 s after task failure.

Knee-Extensor Force

Participants sat upright on a custom-built chair with the knees and hips at 90° of flexion and were secured using straps across trunk and shoulders. Knee-extensor force was measured using a calibrated load cell (Tedea Huntleigh 615, Vishay, Basingstoke, UK) positioned directly behind the point of applied force and connected to a noncompliant cuff attached around the participant's right leg, 1 - 2 cm superior to the ankle malleoli. Following a set of preparatory contractions (2 x 50%, 2 x 75% and a minimum of 2 x 100% 'efforts'), MVCs were performed for 3 - 5 s with strong verbal encouragement and visual feedback of force. MVC force was measured as the highest 500-ms plateau.

Electrical Stimulation of the Femoral Nerve

Single electrical stimuli (200 μ s pulse width) were delivered to the right femoral nerve via 5 cm² surface electrodes (CF5050B, Nidd Valley Medical, Hampshire, UK) and a constant-current stimulator (DS7AH, Digitimer Ltd, Hertfordshire, UK). The cathode was positioned over the femoral 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 highest mechanical twitch force and peak-to-peak M-wave amplitude in the *vastus lateralis* (VL) was located. Stimulations were delivered at increasing intensity (10 mA + 20 mA) until no further increase in either twitch force or M-wave amplitude could be elicited. The plateau intensity was increased by 30% to ensure supramaximality (254 $\pm$ 53 mA). Femoral nerve stimulation (FNS) was delivered during and within 2 s of MVCs to quantify M-waves, potentiated twitch force ($Q_{tw,pot}$) and VA_{FNS} . VA_{FNS} was calculated using the interpolated twitch technique (ITT) where the amplitude of the

superimposed twitch (SIT) was normalized to the corresponding $Q_{tw,pot}$ using the equation $VA_{FNS}(\%) = (1 - (SIT/Q_{tw,pot})) \times 100$ (7).

Transcranial Magnetic Stimulation

Single TMS pulses (1-ms duration) were delivered with a 110-mm diameter concave double-cone coil powered by a mono-pulse magnetic stimulator (Magstim 200², The Magstim Company Ltd, Whitland, UK) with a postero-anterior intracranial current flow. Optimal coil position (location eliciting the largest motor evoked potential (MEP) in the VL and a concurrent small MEP in the *biceps femoris* (BF) with stimulations delivered at 70% maximal stimulator output (MSO)) was measured relative to the vertex and clearly marked on the scalp with indelible ink. Resting motor threshold (rMT) was determined at the beginning of each TTF visits using the modified relative frequency method (20), defined as the intensity that elicited a VL MEP of ≥ 0.05 mV in three out of six trials (42). Stimulations were delivered at 130% rMT (18) which corresponded to $70 \pm 11\%$ MSO (VL MEP/ M_{sup} area of 60 – 70% at 50 - 75% MVC). The BF MEP response was $< 20\%$ of the VL response at all contraction strengths. Participants performed three sets of contractions (100%, 75% and 50% MVC, separated by 10 s rest) with 20-s rest between sets (Figure 1C). The estimated resting twitch (ERT) was estimated by taking the y-intercept of a linear regression (baseline: $r = 0.95 \pm 0.04$; fatigued state: $r = 0.95 \pm 0.05$) of the SIT-voluntary force relationship (43). VA_{TMS} was subsequently quantified using the equation $VA_{TMS}(\%) = (1 - (SIT/ERT)) \times 100$. Where regressions were not adequately linear (defined as $r < 0.85$ (16)), or an individual set or contraction was problematic, it was excluded and the mean of the two sets was used to estimate ERT. This occurred in $< 5\%$ of evaluations.

Electromyographic Responses

Surface electromyography (EMG) was recorded from the right VL and the lateral head of the BF. The skin was shaved, abraded and cleaned with an alcohol wipe. Two single-use Ag/AgCl electrodes (33 x 22 mm, Kendall H59P, Mansfield, MA, USA) were placed in a bipolar configuration (inter-electrode distance of 20 mm) over the muscle belly. The reference electrode was placed over the patella. Electrode placement was marked with indelible ink to ensure consistent positioning between visits. Raw EMG signal was amplified (x 1000), digital band-pass filtered (20 Hz – 2 kHz) and sampled at 4 kHz. The peak-to-peak amplitude and area under the curve of the VL $M_{\max}$ and $M_{\sup}$, and VL and BF MEP at each contraction strength were quantified. VL MEPs were normalised to the corresponding maximal M-wave at rest ($M_{\max}$) or during an MVC ($M_{\sup}$). The cortical silent period (CSP) was measured during three MVCs from stimulus artefact to the continuous resumption of EMG, determined by the same experimenter using visual inspection of the EMG trace.

Statistical Analyses

Data are presented as mean $\pm$ SD throughout. All statistical procedures were completed using IBM SPSS v22. Prior to ANOVA, homogeneity of variance was confirmed using Levene's test. Data were checked for sphericity using Mauchly's test and if violated, the Greenhouse-Geisser correction was applied. Two-way mixed ANOVA were performed to determine differences in HR, S_pO_2 and RPE during the intervention protocol (exposure $\times$ group) and differences in exercise time and HBmass before and after the intervention (trial $\times$ group). For neuromuscular and within-exercise data, three-way mixed design ANOVA were performed (trial $\times$ time $\times$ group), where trial denotes TTF_{Pre} vs. ISO. For within exercise data, a resting baseline, minute 1

– 4 and the final minute of constant-power cycling were included in the statistical analysis given the duration of the shortest exercise time. Following a significant overall interaction, two-way repeated measures ANOVA were performed in each group (trial $\times$ time). To account for the difference in exercise time after the IH intervention, two-way repeated measures ANOVA were performed in the IH group alone (trial $\times$ time), where trial denotes TTF_{-Pre} vs. TTF_{-Post}. Following a significant interaction effect, post-hoc analysis was conducted using Tukey's HSD tests to localize differences. Statistical significance was set at $p < 0.05$. Effect sizes are presented as partial eta squared (η_p^2) for main and interaction effects and Cohen's d_{av} for pairwise comparisons.

RESULTS

Exercise Time (Figure 2)

Following an interaction of trial $\times$ group, exercise time did not differ significantly before and after the control intervention (535 ± 124 vs. 557 ± 131 s, $p > 0.05$; $d = 0.18$), but improved by $35 \pm 18\%$ after the IH protocol (535 ± 213 to 713 ± 271 s, $p < 0.05$; $d = 0.73$).

Haematological Measures

Due to a technical issue, nine participants in the IH group and four participants in the control group completed the post-intervention oCO_r measurement 72 h after the final exposure. No differences were found for any Hbmass, plasma volume, blood volume or [Hb] before and after the intervention (all $p < 0.05$) (see Table, Supplemental Digital Content 1, Haematological measures before and 72-h after an intervention of intermittent hypoxia (IH) or control protocol in normoxia, <http://links.lww.com/MSS/A993>).

Maximal Voluntary Force

Following an interaction of trial $\times$ time $\times$ group, MVC was reduced from pre- to post-exercise in the control group (time: $p < 0.001$; $\eta_p^2 = 0.92$), with no differences before and after the intervention (trial $\times$ time: $p = 0.69$; $\eta_p^2 = 0.02$). The exercise-induced decrease in MVC in the IH group was alleviated at isotime after the intervention (trial $\times$ time: $p < 0.001$; $\eta_p^2 = 0.78$, post-exercise MVC: $p < 0.05$; $d = 0.67$). When cycling continued to task failure in TTF-Post, the resulting decrease in MVC (Figure 3) did not differ before and after the intervention (trial $\times$ time: $p = 0.16$; $\eta_p^2 = 0.19$).

Central Fatigue and Corticospinal Excitability

Following an interaction of trial $\times$ time $\times$ group, VA_{TMS} was reduced post-exercise in the control group (time: $p < 0.001$; $\eta_p^2 = 0.92$), with no differences before and after the intervention (trial $\times$ time: $p = 0.52$; $\eta_p^2 = 0.06$). As presented in Figure 3, the exercise-induced decrease in VA_{TMS} (i.e. central fatigue), was alleviated at isotime after the IH intervention (trial $\times$ time: $p = 0.012$; $\eta_p^2 = 0.49$, post-exercise VA_{TMS} : $p < 0.05$; $d = 1.20$). When cycling continued to task failure, the decrease in VA_{TMS} did not differ before and after the intervention (trial $\times$ time: $p = 0.296$; $\eta_p^2 = 0.11$). The overall pattern in regards to VA_{FNS} was analogous to that described for VA_{TMS} (Table 1). No differences were found for corticospinal excitability parameters (all $p > 0.05$) and data for 100% MVC are presented in Table 1.

Peripheral Fatigue and Neuromuscular Transmission

Following an interaction of trial $\times$ time $\times$ group, $Q_{tw,pot}$ was reduced post-exercise in the control group (time: $p < 0.001$; $\eta_p^2 = 0.93$), and the level of peripheral fatigue did not differ after the

normoxic protocol (trial $\times$ time: $p = 0.053$; $\eta_p^2 = 0.44$). In contrast, the exercise-induced decrease in $Q_{tw,pot}$ was alleviated at isotime after the IH intervention (trial $\times$ time: $p = 0.004$; $\eta_p^2 = 0.57$, post-exercise $Q_{tw,pot}$: $p < 0.05$; $d = 0.94$). However, the level of peripheral fatigue reached at task failure did not differ before and after the IH intervention (trial $\times$ time: $p = 0.079$; $\eta_p^2 = 0.28$). No differences were found for parameters relating to neuromuscular transmission (all $p > 0.05$) and data are presented in Table 1.

Within-Exercise Measures

Data for HR, RPE, breathlessness and pulmonary gas exchange are presented in Table 2.

Pulmonary Ventilation

Following an interaction of trial $\times$ time $\times$ group, $\dot{V}_E$ was found to be higher at isotime following the IH intervention ($p < 0.05$; $d = 0.27$). A higher $\dot{V}_E$ was also observed in TTF_{-Pre} vs. TTF_{-Post} ($p = 0.004$; $\eta_p^2 = 0.42$), such that $\dot{V}_E$ was $17 \pm 21\%$ higher at task failure after the intervention ($p < 0.05$; $d = 0.43$).

Hemoglobin Oxygen Saturation

Following an interaction of trial $\times$ time $\times$ group, the profile of decreasing S_pO_2 during exercise in severe hypoxia (Figure 4) did not differ before and after the intervention in the control group (trial $\times$ time: $p = 0.45$; $\eta_p^2 = 0.12$). This is in contrast to the IH group (trial $\times$ time: $p = 0.001$; $\eta_p^2 = 0.35$), where S_pO_2 was higher at isotime after the intervention ($p < 0.05$; $d = 1.57$). However, when exercise time was prolonged to task failure, end-exercise S_pO_2 reached the same level as in TTF_{-Pre} ($p > 0.05$; $d < 0.1$).

Arterial Oxygen Content

Following an interaction of trial $\times$ time $\times$ group, in the control group, the profile of C_aO_2 did not differ before and after the intervention (trial $\times$ time: $p = 0.741$; $\eta_p^2 = 0.08$). This is in contrast to the IH group (trial $\times$ time: $p < 0.001$; $\eta_p^2 = 0.36$), where C_aO_2 was higher at isotime: 16.8 ± 1.1 vs. $15.9 \pm 0.8 \text{ mL O}_2 \cdot \text{dL}^{-1}$; $p < 0.05$; $d = 0.90$). However, at task failure, although the interaction of trial $\times$ time was significant ($p = 0.041$; $\eta_p^2 = 0.20$) no differences were found when post-hoc analysis was conducted.

Cerebral Blood Flow Velocity and Cerebral Oxygen delivery

Due to an inadequate signal, one participant was removed from the analysis of MCA_v and the derived estimate of $C\dot{D}O_2$ (IH $n = 11$, control $n = 7$). No significant differences were found in the profile of MCA_v or $C\dot{D}O_2$ (i.e. interaction effects) before and after the interventions (all $p > 0.05$). Data are presented in Figure 4.

The Intervention Protocols

No incidence of AMS (LLQ score ≥ 3) was observed. Following an interaction of exposure $\times$ group, in the IH group, resting S_pO_2 improved during exposure 9 and 10 (e.g. $79 \pm 6\%$ vs. $83 \pm 4\%$ for exposure 1 vs. 10, $p < 0.05$; $d = 0.59$). Exercising S_pO_2 in IH improved from the first to the last exercise bout (74 ± 4 vs. 78 ± 4 , $p < 0.05$; $d = 1.0$). S_pO_2 was $98 \pm 1\%$ at rest and $97 \pm 1\%$ during exercise in the control group and did not differ over exposures 1 to 10 (all $p > 0.05$). No differences were found between groups in exercising HR or RPE (all $p > 0.05$). During the first session of exercise, HR was $131 \pm 15 \text{ b} \cdot \text{min}^{-1}$ and $131 \pm 6 \text{ b} \cdot \text{min}^{-1}$ in IH and control, respectively. By exposure 10, exercising HR reduced to $122 \pm 14 \text{ b} \cdot \text{min}^{-1}$ and $124 \pm 5 \text{ b} \cdot \text{min}^{-1}$ in

IH and control, respectively. In both groups, RPE was 12 ± 1 on exposure 1, and 11 ± 1 by exposure 10.

DISCUSSION

The aim of the present study was to assess both whole-body exercise tolerance and the mechanisms of neuromuscular fatigue in severe hypoxia following an IH protocol. Exercise tolerance in severe hypoxia improved following an IH protocol completed in the same severity of hypoxia, but not in a control group who completed a matched protocol in normoxia. This is the first study to show that the development of neuromuscular fatigue with whole-body exercise in severe hypoxia is attenuated following an IH protocol. In particular, central fatigue was lower at the same exercise time achieved prior to the IH intervention.

The IH Protocol and Exercise Tolerance in Severe Hypoxia

The IH protocol involved ten exposures to a low P_{iO_2} (82 mmHg) at rest (2-hour duration) with 30 minutes of moderate-intensity exercise ($25\% \dot{W}_{peak}$) within seven of the sessions. Selecting an optimal hypoxic dose that is both suitable for practical application and capable of eliciting beneficial adaptations is challenging because protocols vary considerably in the literature depending on the aim of the specific study. Nevertheless, in the design of the present study, we considered the characteristics of IH protocols (in relation to the combination of exposure and training, the training intensity and duration and the total hypoxic dose) and exercise performance in severe hypoxia as reviewed by Muza *et al* in 2007 (28) and investigated in later studies (e.g. (6,7,9,10,24)). In the initial IH sessions of the present study, there was an anticipated and pronounced arterial hypoxemia at rest ($S_pO_2 < 80\%$), which improved in the last two sessions (~

83%). During the first exercise bout in IH, S_pO_2 decreased further, to $< 75\%$. This improved significantly by the final exercise bout alone ($\sim 78\%$). Comparable improvements in S_pO_2 during hypoxic exercise have been reported during constant-power exercise following an IH protocol (e.g. (7)), although not consistently (6).

Constant-power cycling in AH resulted in task failure in 8.9 ± 2.9 min. During the intervention, power output was not equivalent in IH and control due to the increase in relative exercise intensity at the same absolute power output in severe hypoxia. To detect changes due to the intervention, the exercise intensity in normoxia was adjusted to match heart rate recorded during the first exercise bout in the IH group ($\sim 131 \text{ b} \cdot \text{min}^{-1}$). In matching the cardiovascular demand of the exercise, it is noted that exercise was also performed at the same perceived exertion (RPE 12), where S_pO_2 was significantly greater for the control group (98%). It was therefore hypoxic exposure and/or hypoxic exercise that elicited an improvement in exercise tolerance in severe hypoxia in the IH group. Although adaptations cannot be attributed to one of these stressors alone, seven sessions of matched-intensity exercise in normoxia did not improve exercise tolerance in severe hypoxia. Indeed, there was no statistical difference from baseline exercise time in the control group, where the mean difference (36 s) was deemed too small to warrant, and justify from an ethical standpoint, a further trial in severe hypoxia. The improvement in exercise time in the IH group was substantial ($\sim 35\%$) and systematic (range: 1.1 – 5.2 min). Given the successful blinding to the intervention and the blinding of all real-time feedback during the cycling trials, we consider it to be highly unlikely that the improvement in exercise tolerance was due to an anticipated benefit of IH (i.e. a placebo effect).

Neuromuscular Fatigue in Severe Hypoxia

Constant-power cycling induced neuromuscular fatigue in AH, indicated by a ~ 20% decrease in the ability to produce maximal voluntary force in the knee extensors. After the IH protocol, the reduction in MVC force was less pronounced at isotime (~ 12%), but reached pre-intervention levels when exercise continued to task failure. One of the primary limitations to whole-body exercise tolerance in severe hypoxia is exacerbated central fatigue (4,17). The decrease in VA_{TMS} from pre- to post-exercise in severe hypoxia prior to the intervention was similar to that reported previously in AH (17,19). Goodall *et al* (19) found a 12% decrease in VA_{TMS} following constant-power cycling in AH. After acclimatization, central fatigue was alleviated at isotime and this was attributed, in part, to an improved cerebral O_2 availability. However, due to the constraints of the wider project protocol, an improvement in exercise tolerance was not confirmed. In the present study, the IH protocol resulted in an alleviated central fatigue at isotime, where the decrease from baseline was no longer significant. When exercise was permitted to continue to task failure after the IH intervention, central fatigue (and indeed the overall decrease in MVC force) ultimately reached the same levels that coincided with task failure before the intervention (12% decrease in VA_{TMS}). We therefore propose that the alleviation of the central contribution to neuromuscular fatigue is an important, though not necessarily the singular, mechanism by which exercise was prolonged following the IH protocol.

The mechanisms for the alleviation of central fatigue may be related to improved cerebral oxygenation secondary to an improved $\dot{C}DO_2$ (11). A number of researchers have substantiated a link between a reduced $\dot{C}DO_2$ and the impairment to whole-body exercise that occurs in severe hypoxia (e.g. (48)). However, a challenge in the research area is isolating the influence of a

systemic improvement in oxygenation (i.e. SpO_2) from an improvement in cerebral oxygenation. Isolating the effect of systemic and cerebral oxygenation requires innovative experimental procedures such as CO_2 clamping, which increases CBF and therefore $C\dot{D}O_2$. Interestingly, increasing $C\dot{D}O_2$ in AH has not been shown to improve exercise tolerance (12). However, the method is problematic during whole-body exercise (e.g. due to increased respiratory muscle work), and has only been performed with a neuromuscular assessment in a single-limb model (36). In the present study, although the estimate of C_aO_2 was higher at isotime after the IH intervention (due to the improvement in S_pO_2 and not $[Hb]$), CBF and $C\dot{D}O_2$ were not, despite the improvement in exercise time. The relationship between $C\dot{D}O_2$, exercise tolerance and central fatigue in severe hypoxia remains unresolved (39). However, the eventual use of O_2 in mitochondrial oxidation depends not only on $C\dot{D}O_2$ but on the capillary O_2 tension, the O_2 conductance from capillary to mitochondria and the cerebral metabolic rate of O_2 (31). Under normoxic conditions there is a tight coupling of the cerebral metabolic rate of O_2 and CBF (30). During physiological increases in neuronal activity (e.g., synaptic transmission and firing rate), there is an uncoupling of these variables such that CBF largely exceeds the consumption of O_2 in tissue (13). It may be that the signal for reduced central motor output depends on a step that is uncoupled with $C\dot{D}O_2$ during whole-body exercise in severe hypoxia, and this may be altered with an IH protocol. However, this is speculative and further studies on the relationship between cerebral O_2 metabolism and central fatigue in severe hypoxia are warranted.

Neither the CSP, as a representation GABA(B) receptor-mediated inhibition of cortical excitability (40), or the MEP, used to assess changes in the state of excitability in the corticospinal system (8), were modulated pre- to post-exercise exercise in severe hypoxia, or by

the intervention itself. Previous findings indicate time-dependent increases in corticospinal excitability (at rest) with severe hypoxia (3,35). However, both studies used continuous exposures (3 h and 14 days, respectively) and it may be that the discontinuous nature of an IH protocol (i.e. the wash-out in normoxia) masked any transient neurophysiological alterations over the time-course of an IH protocol. This warrants further investigation, given the therapeutic potential of IH (29,46).

As evidenced by an increase in $\dot{V}_E$ and decrease in $P_{ET}CO_2$ following the IH intervention, the IH protocol used conferred a level of ventilatory adaptation to hypoxic exercise. This resulted in an increase in S_pO_2 during constant-power cycling following the IH protocol. A proposed threshold of arterial hypoxemia where hypoxia-sensitive mechanisms originating in the CNS are thought to override other inhibitory influences on central motor output (i.e. afferent feedback from the exercising limb) during whole-body exercise, is below 70-75% (4,11). We note that at isotime following the IH intervention, S_pO_2 was 79% (vs. 70% at task failure in TTF-Pre). A number of previous studies have shown similar ventilatory adaptations with IH which resulted in an increased $\dot{V}_E$ and S_pO_2 during hypoxic exercise, augmented by increased hypoxic chemosensitivity (7,10,28). Limited studies have investigated exercise tolerance in severe hypoxia, but some have shown improvements in cycle time-trial performance comparable to CH (5,7). More recently, a study that used four exposures of 4 h to $P_{iO_2} \approx 92$ mmHg, increased exercise $\dot{V}_E$ and S_pO_2 were observed without changes in cerebral oxygenation or constant-power cycling to task failure in hypoxia (10). A beneficial effect of IH on exercise tolerance in severe hypoxia is not a consistent finding (6,9), and the disparity may be due to differences relating to protocol and hypoxic dose.

In the present study, we found no change in Hbmass, plasma volume or [Hb]. Hbmass is a measure of O₂ carrying capacity that is not subject to vascular volume shifts. A recent study reported an increase in Hbmass following an IH protocol in moderate hypoxia (34). This is surprising given the evidence suggesting that the total duration of hypoxic exposure required to produce a change in Hbmass is equivalent to more than ~ 7 d continuous exposure (32).

The Peripheral Contribution to Neuromuscular Fatigue

In AH, peripheral locomotor muscle fatigue was identified as a decrease in potentiated quadriceps twitch force by ~ 20%. The results of earlier studies indicate that in the severe-intensity domain, the level of peripheral fatigue at task failure in AH is less than that reached at task failure in normoxia (> 30%) (e.g. (4)). In severe hypoxia, task disengagement occurs before metabolic disturbance reaches levels attained at the end of the same task performed in normoxia. For this reason, we do not consider peripheral fatigue to be a major limitation to whole-body exercise under these specific and extreme conditions. Nevertheless, following the IH protocol, the decrease in $Q_{tw,pot}$ was less prominent at isotime (~ 16%). This is in contrast to the findings following a 14-day exposure to high altitude, where the development of peripheral fatigue was not alleviated (3). The mechanisms for this are equivocal but may be related to a lower limb blood flow and therefore limb O₂ delivery, an important regulator of peripheral fatigue (2). Speculatively, in the present study, an unchanged limb blood flow coupled with an increase in C_aO_2 is one explanation for the reduction in peripheral fatigue. Alternatively, an exercise $\times$ hypoxia interaction may have induced skeletal muscle adaptations (21) that delayed the development of peripheral fatigue such that it was lower at isotime, but ultimately reached the same levels at task failure.

The decision to disengage from a task can be described as an internally coordinated response to internal and/or external stimuli, i.e. a behaviour (23). It would be simplistic to ascribe this solely to lower-level neurophysiological properties (22). The perception of the sensations associated with hypoxic exercise is an important consideration, and both perceived limb discomfort and breathlessness (notably, in spite of a higher $\dot{V}_E$), were also lower at isotime. We further acknowledge that task failure in severe hypoxia is likely to have a cognitive component (44). Disentangling the relative contributions of these complex and interactive processes is a major challenge for exercise scientists and in hypoxic physiology, warrants further consideration.

In summary, the novel findings of this study were that whole-body exercise tolerance in severe hypoxia was prolonged following a protocol of intermittent hypoxia involving exposure and exercise, but not in a control group who performed a matched protocol in normoxia. At isotime following the IH intervention, the central contribution to neuromuscular fatigue was alleviated. These alterations occurred alongside an augmented ventilatory response to hypoxic exercise which improved the pronounced arterial hypoxemia induced by hypoxic exercise in the severe-intensity domain.

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CONFLICTS OF INTEREST AND SOURCE OF FUNDING

No conflicts of interest, financial or otherwise, are declared by the authors. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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FIGURE CAPTIONS

Figure 1. Overview of the experimental protocol (A), the constant-power cycling trial visits (B) and the neuromuscular assessment (C). Fam¹ and Fam², familiarisation sessions one and two; Max, measurement of maximal O₂ uptake and peak power; oCO₂, optimised carbon monoxide rebreathing method; TTF, trial to task failure; ISO, constant-power cycling to isotime; rMT, resting motor threshold; FNS, femoral nerve stimulation; TMS, transcranial magnetic stimulation. *Denotes exposures on which 30 min exercise was performed. †Indicates that this trial did not differ from TTF_{Pre} and was treated as an isotime trial.

Figure 2. Time to task failure (s) with constant-power cycling in severe hypoxia before (Pre) and after (Post) a protocol of intermittent hypoxia (IH) or a matched protocol in normoxia (Control). * $p < 0.05$, Pre vs. Post. The mean is plotted as the closed circle.

Figure 3. Neuromuscular measures made Pre- (open bars) and Post- (closed bars) constant-power cycling in severe hypoxia, before and after a protocol of intermittent hypoxia (IH, left column) or a matched protocol in normoxia (Control, right column). MVC, maximal voluntary contraction; VA_{TMS}, cortical voluntary activation; Q_{tw,pot}, potentiated quadriceps twitch force; TTF, time to task failure. * $p < 0.05$ vs. Post. † $p < 0.05$, Post-exercise in TTF_{Pre}.

Figure 4. Hemoglobin oxygen saturation (S_pO_2 , top row), cerebral blood flow velocity (MCA_v , middle row) and cerebral oxygen delivery index (CDO_2 , bottom row) in the intermittent hypoxia group (IH, left column) and control group (right column) during TTF-Pre (open circles) and isotime (grey circles). * $p < 0.05$ vs. ISO.

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Figure 1

Figure 2

Figure 3

Figure 4

Table 1. Neuromuscular parameters pre and post constant-power cycling to task failure, before and after a protocol of intermittent hypoxia (IH) or a matched control protocol in normoxia.

		TTF _{-Pre}		ISO		TTF _{-Post}	
		Pre	Post	Pre	Post	Pre	Post
M_{max} amplitude (mV)	IH	6.2 ± 1.9	5.6 ± 1.6	6.5 ± 3.2	6.1 ± 3.5	5.7 ± 2.2	5.8 ± 1.9
	CON	6.0 ± 2.6	4.9 ± 2.4	5.9 ± 2.4	5.3 ± 2.0		
M_{max} area (mV.ms⁻¹)	IH	40.5 ± 11.6	40.8 ± 11.9	44.2 ± 10.1	39.3 ± 17.4	37.4 ± 16.0	42.1 ± 16.2
	CON	40.4 ± 16.4	34.4 ± 19.1	40.5 ± 15.3	40.1 ± 16.9		
VA_{FNS} (%)	IH	92 ± 3	85 ± 4*	93 ± 5	90 ± 4†	93 ± 3	86 ± 3*
	CON	93 ± 3	86 ± 3*	92 ± 3	85 ± 5*		
M_{sup} amplitude (mV)	IH	6.2 ± 2.1	5.8 ± 1.6	7.3 ± 3.3	6.6 ± 3.6	6.3 ± 2.1	5.8 ± 1.9
	CON	7.4 ± 2.1	6.0 ± 3.0	7.1 ± 2.7	5.5 ± 2.4		
M_{sup} area (mV.ms⁻¹)	IH	41.1 ± 11.5	36.7 ± 8.1	45.4 ± 16.0	41.5 ± 18.1	43.0 ± 12.4	36.5 ± 12.9
	CON	38.3 ± 11.0	39.7 ± 21.4	37.4 ± 11.5	32.6 ± 15.7		
MEP/M_{sup} amplitude	IH	0.53 ± 0.12	0.50 ± 0.13	0.46 ± 0.13	0.48 ± 0.15	0.50 ± 0.18	0.54 ± 0.14
	CON	0.40 ± 0.10	0.46 ± 0.12	0.50 ± 0.14	0.52 ± 0.07		
MEP/M_{sup} area	IH	0.63 ± 0.15	0.66 ± 0.15	0.56 ± 0.10	0.61 ± 0.17	0.59 ± 0.13	0.71 ± 0.11
	CON	0.59 ± 0.10	0.58 ± 0.12	0.67 ± 0.13	0.73 ± 0.16		
CSP (ms)	IH	169 ± 46	156 ± 45	179 ± 43	169 ± 50	183 ± 47	172 ± 48
	CON	179 ± 85	176 ± 77	174 ± 74	164 ± 70		
ERT/MVC (%)	IH	21 ± 10	14 ± 5	20 ± 8	13 ± 4	20 ± 8	14 ± 6
	CON	25 ± 8	15 ± 7	21 ± 10	13 ± 8		

MVC, maximal voluntary contraction; M_{max}, maximal muscle compound action potential; M_{sup}, superimposed maximal muscle compound action potential; MEP, motor evoked potential, CSP, cortical silent period; ERT, estimated resting twitch. **p* < 0.05 vs. pre-exercise. †*p* < 0.05 vs. post-exercise in TTF_{-Pre}.

Table 2. Within-exercise data at rest (following a 15 min wash-in period to severe hypoxia) and in the final minute of exercise during TTFs before and after an intermittent hypoxia protocol or a matched control protocol in normoxia.

			TTF-Pre	ISO	A	TTF-Post	B
$\dot{V}_E$ (L·min ⁻¹)	IH	Rest	16.7 ± 5.8	17.7 ± 6.0	† [#]	18.3 ± 6.5	† [#]
		End-Ex	145.1 ± 17.9	150.1 ± 18.5	*	158.8 ± 21.9	*
	CON	Rest	17.1 ± 4.8	18.9 ± 5.0			
		End-Ex	151.2 ± 16.3	150.9 ± 16.8			
$P_{ET}CO_2$ (mmHg)	IH	Rest	38.1 ± 4.8	33.8 ± 2.2	† ^{#*}	34.4 ± 3.2	† [#]
		End-Ex	28.1 ± 4.2	27.1 ± 3.9		24.7 ± 2.6	
	CON	Rest	33.7 ± 2.8	33.9 ± 3.4			
		End-Ex	25.1 ± 2.4	25.1 ± 2.0			
$\dot{V}O_2$ (L·min ⁻¹)	IH	Rest	0.52 ± 0.14	0.49 ± 0.15	†	0.50 ± 0.15	†
		End-Ex	2.42 ± 0.48	2.29 ± 0.32		2.41 ± 0.28	
	CON	Rest	0.52 ± 0.17	0.57 ± 0.12			
		End-Ex	2.49 ± 0.29	2.47 ± 0.46			
$\dot{V}CO_2$ (L·min ⁻¹)	IH	Rest	0.62 ± 0.16	0.60 ± 0.18	†	0.61 ± 0.20	†
		End-Ex	3.60 ± 0.37	3.38 ± 0.33		3.34 ± 0.35	
	CON	Rest	0.61 ± 0.09	0.66 ± 0.16			
		End-Ex	3.47 ± 0.31	3.47 ± 0.37			
$\dot{V}_E/\dot{V}CO_2$ (L·min ⁻¹)	IH	Rest	26.48 ± 4.00	29.57 ± 263	†	29.69 ± 2.20	† ^{#*}
		End-Ex	40.54 ± 4.57	42.02 ± 6.33		47.44 ± 3.34	*
	CON	Rest	27.57 ± 4.64	28.89 ± 3.02			
		End-Ex	43.60 ± 2.70	43.57 ± 4.34			
HR (b·min ⁻¹)	IH	Rest	84 ± 6	80 ± 3	†	82 ± 5	†
		End-Ex	177 ± 6	172 ± 8		175 ± 7	
	CON	Rest	84 ± 9	85 ± 7			
		End-Ex	173 ± 8	175 ± 7			
RPE	IH	End-Ex	20 ± 0	17 ± 2	† ^{#*}	20 ± 0	†
	CON	End-Ex	20 ± 0	20 ± 0			
Dyspnoea	IH	End-Ex	10 ± 0	6 ± 2	† ^{#*}	10 ± 0	†
	CON	End-Ex	10 ± 0	10 ± 0			

$\dot{V}_E$, minute ventilation; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; $\dot{V}O_2$, oxygen uptake, $\dot{V}CO_2$, carbon dioxide production; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; HR, heart rate; RPE, rating of perceived exertion. **A**, TTF-Pre vs. ISO; **B**, TTF-Pre vs. TTF-Post in IH. [#] $p < 0.05$ main effect of trial; † $p < 0.05$ main effect of time, * $p < 0.05$ vs. TTF¹.

LIST OF SUPPLEMENTAL DIGITAL CONTENT

Supplemental Digital Content 1.doc—Haematological measures before and 72-h after an intervention of intermittent hypoxia (IH) or control protocol in normoxia

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Supplemental Digital Content

Haematological measures before and 72-h after an intervention of intermittent hypoxia (IH) or control protocol in normoxia.

		Pre-Intervention	Post-Intervention	$f_{(1,7)}$	p	np^2
Total Haemoglobin Mass (g)	IH	875 ± 155	880 ± 112	0.03	0.857	0.03
	Control	860 ± 117	860 ± 104			
Plasma Volume (mL)	IH	937 ± 259	899 ± 188	0.14	0.580	0.13
	Control	922 ± 127	914 ± 97			
Blood Volume (mL)	IH	1812 ± 400	1778 ± 1281	0.18	0.683	0.01
	Control	1781 ± 244	1774 ± 165			
Haemoglobin Concentration (g·dL ⁻¹)	IH	16.5 ± 1.0	16.5 ± 1.0	0.51	0.490	0.04
	Control	15.0 ± 0.1	15.4 ± 0.5			

IH, intermittent hypoxia. IH, $n=9$; control, $n=4$. ANOVA result is provided for the interaction of trial (pre- and post-intervention) x group (IH vs. control).