Eur J Appl Physiol
DOI 10.1007/s00421-017-3669-y

ORIGINAL ARTICLE

Effects of high-altitude exposure on supraspinal fatigue and corticospinal excitability and inhibition

Mathieu Marillier[1,2] · Pierrick J. Arnal[3] · Thibault Le Roux Mallouf[1,2] · Thomas Rupp[1,2,4] · Guillaume Y. Millet[1,2,3,5] · Samuel Verges[1,2]

Received: 1 February 2017 / Accepted: 21 June 2017

Abstract
Purpose While acute hypoxic exposure enhances exercise-induced central fatigue and can alter corticospinal excitability and inhibition, the effect of prolonged hypoxic exposure on these parameters remains to be clarified. We hypothesized that 5 days of altitude exposure would (i) normalize exercise-induced supraspinal fatigue during isolated muscle exercise to sea level (SL) values and (ii) increase corticospinal excitability and inhibition.
Methods Eleven male subjects performed intermittent isometric elbow flexions at 50% of maximal voluntary contraction to task failure at SL and after 1 (D1) and 5 (D5) days at 4350 m. Transcranial magnetic stimulation and peripheral electrical stimulation were used to assess supraspinal and peripheral fatigues. Pre-frontal cortex and biceps brachii oxygenation was monitored by near-infrared spectroscopy.
Results Exercise duration was not statistically different between SL (1095 ± 562 s), D1 (1132 ± 516 s), and D5 (1440 ± 689 s). No significant differences were found between the three experimental conditions in maximal voluntary activation declines at task failure (SL −16.8 ± 9.5%; D1 −25.5 ± 11.2%; D5 −21.8 ± 7.0%; $p > 0.05$). Exercise-induced peripheral fatigue was larger at D5 versus SL (100 Hz doublet at task failure: −58.8 ± 16.6 versus −41.8 ± 20.1%; $p < 0.05$). Corticospinal excitability at 50% maximal voluntary contraction was lower at D5 versus SL (brachioradialis $p < 0.05$, biceps brachii $p = 0.055$). Cortical silent periods were shorter at SL versus D1 and D5 ($p < 0.05$).
Conclusions The present results show similar patterns of supraspinal fatigue development during isometric elbow flexions at SL and after 1 and 5 days at high altitude, despite larger amount of peripheral fatigue at D5, lowered corticospinal excitability and enhanced corticospinal inhibition at altitude.

Keywords Central fatigue · Exercise · Hypoxia · Neuromuscular fatigue

Abbreviations

ANOVA	Analysis of variance
BB	Biceps brachii
BR	Brachioradialis
CSP	Cortical silent period
D1	After 1 day of high-altitude exposure
D5	After 5 days of high-altitude exposure
Db10	Paired stimulation at 10 Hz
Db100	Paired stimulation at 100 Hz
Db10:100	Ratio of Db10 to Db100
EMG	Electromyography
ERT	Estimated resting twitch

Communicated by Nicolas Place.

✉ Samuel Verges
sverges@chu-grenoble.fr

1 U1042, INSERM, Batiment Jean Roget, Faculté de Médecine, La Tronche Cedex, France

2 Laboratoire HP2 (U1042 INSERM), Batiment Jean Roget, Faculté de Médecine, UM Sports Pathologies, Hôpital Sud, Grenoble Alpes University, Avenue Kimberley, 38 434 Echirolles, France

3 Inter-University Laboratory of Human Movement Biology, University of Lyon, University Saint Etienne, Saint-Étienne, France

4 Inter-University Laboratory of Human Movement Biology, University Savoie Mont Blanc, Chambéry, France

5 Human Performance Laboratory, Faculty of Kinesiology, University of Calgary, Calgary, Canada

Published online: 24 June 2017

FiO_2	Fraction of inspired oxygen
HbO_2	Oxyhaemoglobin
HbTot	Total haemoglobin
HHb	Deoxyhaemoglobin
MEP	Motor evoked potential
$MEP{\cdot}Mmax^{-1}$	Motor evoked potential to maximal M-wave ratio
MES	Muscle electrical stimulation
Mmax	Maximal M-wave
MVC	Maximal voluntary contraction
NES	Nerve electrical stimulation
NIRS	Near-infrared spectroscopy
$PetCO_2$	End-tidal CO_2 partial pressure
RPE	Rate of perceived exertion
SIT	Superimposed twitch
SL	Sea level
SpO_2	Arterial oxygen saturation
TB	Triceps brachii
TMS	Transcranial magnetic stimulation
VA_{MES}	Voluntary activation assessed by muscle electrical stimulation
VA_{TMS}	Voluntary activation assessed by transcranial magnetic stimulation

Introduction

Hypoxic exposure is well known to reduce whole-body exercise performance. The severity of arterial hypoxaemia has been shown to influence the contribution of peripheral (i.e., a reduction in force or power output secondary to changes occurring at or distal to the neuromuscular junction) versus central (i.e., a reduction in voluntary activation; Gandevia 2001) fatigue (Verges et al. 2012). It has been suggested that when hypoxemic level increases, a progressive switch occurs from a principally peripheral origin of exercise-induced fatigue to a hypoxia-sensitive central component of fatigue (Amann et al. 2007; Goodall et al. 2010). Supraspinal fatigue, a subset of central fatigue which refers to a failure to generate output from the motor cortex (Gandevia 2001), may explain the greater amount of this central component of fatigue after exercise in severe hypoxia (Goodall et al. 2012).

Most studies about neuromuscular fatigue in hypoxia have investigated the effects of acute (<1 h) hypoxic exposure. To the best of our knowledge, only one research project has explored the effect of several days of hypoxic exposure on exercise-induced peripheral and central fatigues using neuromuscular stimulation (Amann et al. 2013; Goodall et al. 2014). In this experiment, subjects performed a constant-load cycling exercise in three conditions: at sea level (SL; ~130 m of altitude), in acute normobaric hypoxia (FiO_2 0.105) and after 14 days of high altitude acclimatization (5260 m). The power output was set at 50% of SL maximal power output and duration was fixed and equaled the time to exhaustion during acute normobaric hypoxia; therefore, absolute power and duration were the same in the three experimental conditions. Amann et al. (2013) showed that cycling exercise was not sufficient to induce either peripheral or central fatigue at SL, while the same amount of peripheral fatigue was found in acute and prolonged hypoxia. However, acute hypoxia elicited a larger amount of central fatigue (i.e., reduction in voluntary activation level measured with femoral nerve electrical stimulation) compared to prolonged hypoxia. Goodall et al. (2014) reported that supraspinal fatigue assessed in this experiment by transcranial magnetic stimulation (TMS) was induced by exercise in acute hypoxia only. These authors concluded that acclimatization to high altitude does not attenuate the impact of hypoxia on exercise-induced peripheral fatigue, but is able to minimize the development of central fatigue and especially supraspinal fatigue during whole-body exercise.

This study focused on the effect of prolonged hypoxic exposure on neuromuscular fatigue induced by whole-body exercise. However, other studies have investigated the mechanisms of exercise performance limitation in hypoxia by exploring the role of the muscle mass involved during exercise (Calbet et al. 2009; Kayser et al. 1994). Hypoxia consistently reduces whole-body exercise endurance, while isolated muscle exercise performance has been shown to be reduced or similar under hypoxic conditions (e.g., Goodall et al. 2010; Kayser et al. 1994). Whole-body and isolated muscle exercises can differ regarding the effect of hypoxia for two main reasons. First, there is a large reduction in maximal power output during whole-body exercise in hypoxia. As a result, when similar absolute exercise power outputs are used, the relative intensities are different in normoxia versus hypoxia (e.g., Amann et al. 2013; Goodall et al. 2014). The second reason is the difference in terms of contribution of cardiorespiratory mechanisms to exercise limitation that may be larger during whole-body exercise and may induce different arterial desaturation levels during whole-body and isolated muscle exercise for the same inspiratory oxygen partial pressure (Kayser et al. 1994). While acute hypoxic exposure (FiO_2 0.105) reduced leg oxygen delivery and maximal oxygen consumption both during knee extensions and cycling, Calbet et al. (2009) showed that altitude acclimatization (9 weeks at 5260 m) restored systemic and leg O_2 delivery, as well as maximal oxygen consumption, to SL values during knee extensions only. Kayser et al. (1994) compared whole-body and isolated muscle exercise before and after 1 month at 5050 m and suggested that prolonged hypoxic exposure induces a reduced central drive (assessed by EMG recordings) during whole-body exercise only. Hence, the effects of prolonged

hypoxic exposure on central and supraspinal fatigue as assessed by neurostimulation during isolated muscle exercise remain to be elucidated.

Unchanged corticospinal excitability (expressed as motor evoked potential to maximal M-wave ratio, MEP·Mmax^{-1}) and inhibition (expressed as cortical silent period duration, CSP) have been previously reported after acute hypoxic exposure both at rest and after exercise (Goodall et al. 2010; Millet et al. 2012; Rupp et al. 2012), although Szubski et al. (2006) reported reduced resting motor threshold and CSP duration after ~30 min of rest in hypoxia (FiO_2 0.12). Rupp et al. (2012) suggested a time-dependent effect of acute hypoxic exposure (FiO_2 0.12) on corticospinal excitability and inhibition, since increased MEP·Mmax^{-1} and CSP were observed after 3 h but not after 1 h of rest in hypoxia. After 3–5 days at 4554 m, Miscio et al. (2009) reported a significant decrease in the excitability of both excitatory and inhibitory cortical circuits at rest. Goodall et al. (2014) found an increased corticospinal excitability (MEP·Mmax^{-1}) after prolonged stay at high altitude (14 days at 5260 m) compared to SL and acute hypoxic exposure, with no concomitant change in corticospinal inhibition (CSP duration), both before and after whole-body exercise. Hence, the effects of hypoxia and duration of exposure on corticospinal excitability and inhibition both under unfatigued and fatigued conditions need to be clarified.

The aim of the present study was thus to evaluate the effects of 1 and 5 days of high-altitude (4350 m) exposure on peripheral and central determinants of fatigue and on corticospinal excitability and inhibition during isometric elbow flexions to task failure. We hypothesized that (i) short-term (1 day) altitude exposure would enhance the amount of central fatigue during isolated muscle exercise, (ii) acclimatization (characterized here by disappearance of symptoms of acute mountain sickness after 5 days at high-altitude and by significant cerebrovascular adaptations as previously reported by our group (Rupp et al. 2014; Villien et al. 2013) would reduce exercise-induced central fatigue to a level comparable to SL, and (iii) high-altitude exposure would result in an increase in both corticospinal excitability and inhibition (as previously reported by our group after 3 h of hypoxia (Rupp et al. 2012), compared to SL.

Materials and methods

Subjects

Eleven healthy physically active male subjects participated in this study (age 28.2 ± 8.2 years, body mass 71.1 ± 7.4 kg, height 176.4 ± 7.4 cm). All subjects underwent full medical screening and were free of respiratory, cardiovascular, and cerebrovascular diseases. Participants were recreational climbers with no history of severe acute mountain sickness during previous high-altitude ascents. Subjects were unacclimatized to high altitude (no sojourn above 1500 m over the past 3 months). Subjects refrained from prolonged and intense physical activity on the 2 days prior to the tests, abstained from drinking caffeinated beverages on test days, and had their last meal at least 2 h prior to the tests. The study was approved by the local ethics committee (CPP Grenoble Sud Est V) and performed according to the Declaration of Helsinki. Subjects were fully informed of the procedure and risks involved and gave their written consent.

Study design

Subjects performed intermittent isometric elbow flexions to task failure under three experimental conditions: in normoxia at SL (Grenoble, France, altitude 212 m) and after 1 (D1) and 5 (D5) days of altitude exposure at 4350 m (Observatoire Vallot, Mont Blanc, Chamonix, France). D1 and D5 experimental sessions were performed 10.7 ± 3.3 and 14.7 ± 3.3 days after the SL experimental session. Before, during, and after the fatiguing task, neuromuscular evaluations were performed with TMS, muscle, and nerve electrical stimulations (MES and NES, respectively) to assess voluntary activation, motor-cortex excitability, neuromuscular transmission, and muscle contractile properties. Electromyography (EMG) signals of the *biceps brachii* (BB), *brachioradialis* (BR), and *triceps brachii* (TB) muscles were measured continuously. Figure 1 provides an example of typical recordings obtained during these evaluations. In addition, cerebral and muscle (BB) oxygenation was monitored continuously during exercise by near-infrared spectroscopy (NIRS).

Experimental sessions

Subjects sat upright with the right hand supinated and connected to a strain gauge (Captels, St. Mathieu deTreviers, France). Elbow and shoulder angles were set at 90° of flexion. After the initial neuromuscular evaluations (see below), subjects performed a fatiguing task consisting in sets of 19 intermittent submaximal isometric elbow flexions (5-s on/3-s off, total set duration 152 s) interspaced by neuromuscular evaluations (duration 40 s; Fig. 2). Target torque during the fatiguing task was set at 50% of maximal voluntary contraction (MVC). Task failure was defined automatically by a custom designed torque-feedback manager (Labview 8, National Instrument, Austin, TX) when the subject was unable to perform three consecutive contractions adequately, i.e., if three contractions were not at least 4 s of duration or if mean contraction

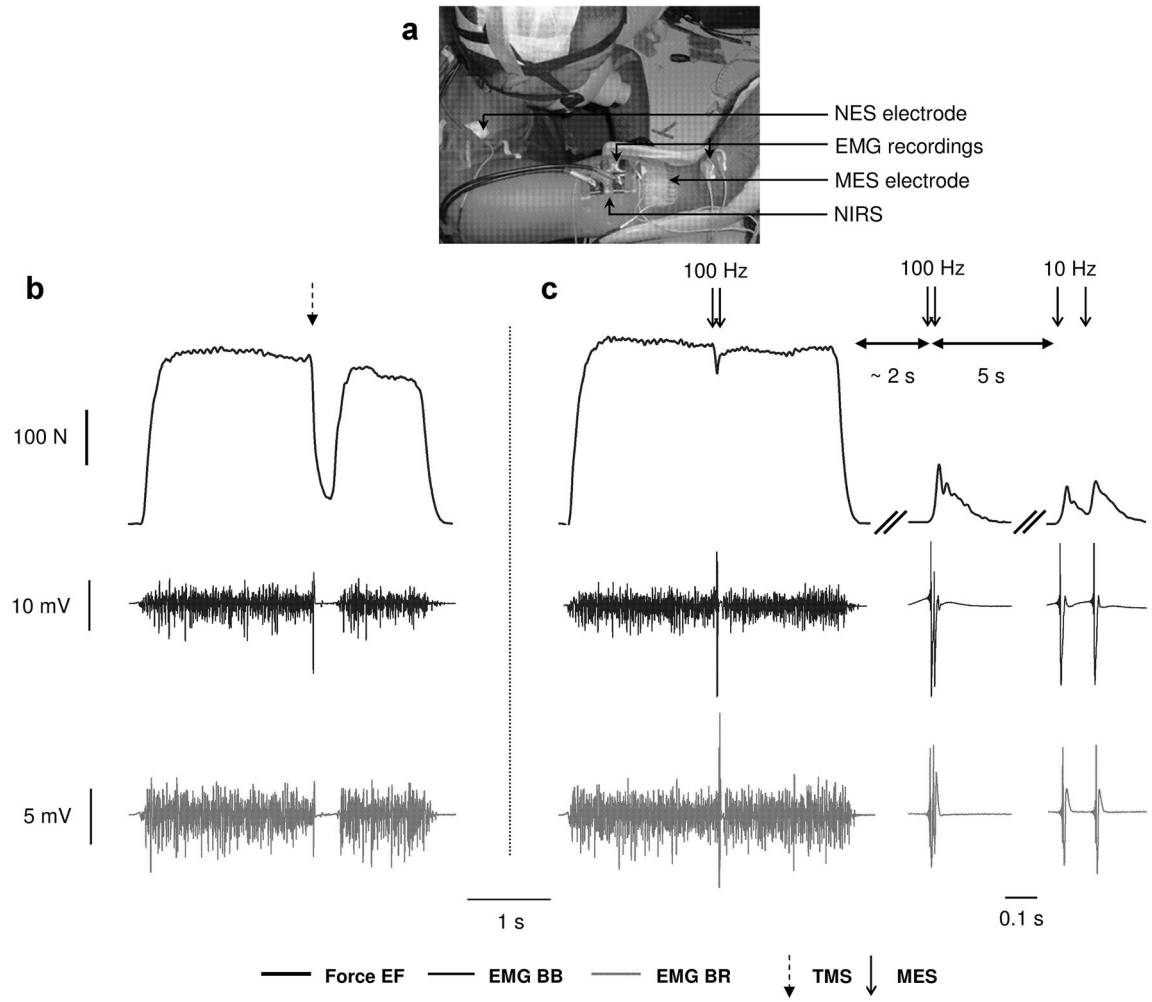

Fig. 1 Experimental setting (**a**) and typical recordings during voluntary, magnetically (**b**) and electrically (**c**) evoked contractions. Torque traces (*black thick line*) during the elbow flexors maximal voluntary contractions, as well as high- and low-frequency doublets (100 and 10 Hz, respectively). EMG is represented with *thin black* (biceps brachii) and *grey* (brachioradialis) *lines*. *Black arrows* indicate the timing of stimulation deliveries. *EF* elbow flexors, *BB* biceps brachii, *BR* brachioradialis, *TMS* transcranial magnetic stimulation, *MES* muscles electrical stimulation, *NIRS* near-infrared spectroscopy

torque was lower than 95% of the target torque. Neuromuscular evaluations were repeated immediately at task failure.

Neuromuscular evaluations

After EMG electrode placement and determination of MES and NES intensity (see below), a standardized warm-up was performed. It consisted of 3 min of repetitions of 5-s isometric elbow flexors contractions/3-s rest with intensities self-adjusted by the subject to progressively reach MVC during the last three trials. Then, the neuromuscular evaluation was performed as summarized in Fig. 2a. The neuromuscular evaluation before the fatiguing task consisted of (I) determination of the optimal TMS site, (II) determination of the optimal TMS intensity (recruitment curve), (III) sets of voluntary contractions to assess cortical voluntary activation (VA_{TMS}), MEP, and CSP as well as NES to assess Mmax (to normalize MEP amplitude), and (IV) supramaximal paired-pulse MES delivered during and 2 s after a MVC to assess peripheral voluntary activation (VA_{MES}) and elbow flexors contractile properties. Every 19 contractions during the fatiguing task, neuromuscular evaluations consisted of one MVC and two submaximal contractions to determine VA_{TMS}, MEP, CSP (as in part III), and supramaximal paired-pulse MES 2 s after the MVC to determine elbow flexors contractile properties (as in part IV). At task failure, neuromuscular evaluation consisted of parts IV and III as before the fatiguing task.

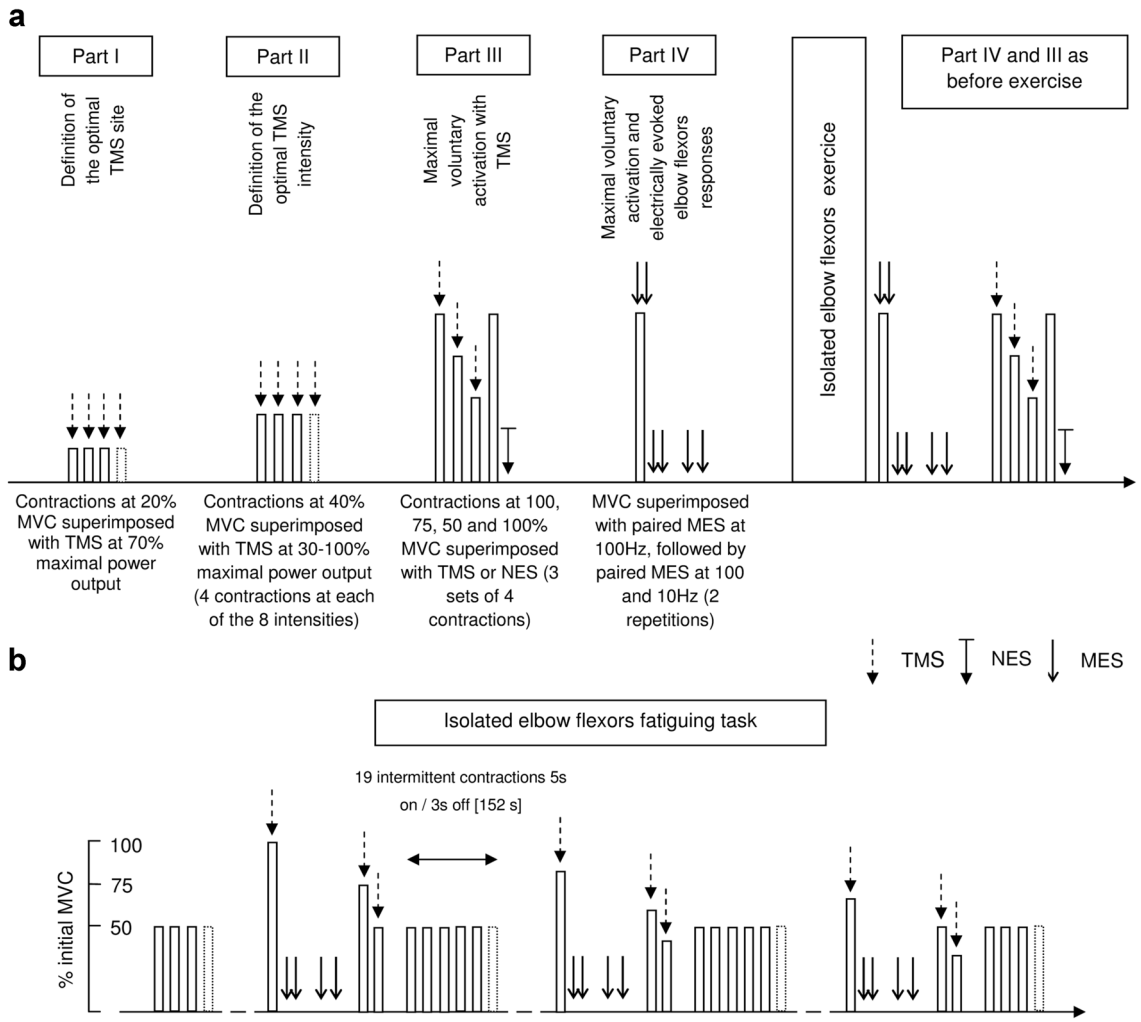

Fig. 2 Overview of the neuromuscular evaluation (**a**) and the isometric elbow flexion fatiguing task (**b**). After a standardized warm-up and maximal voluntary contraction (MVC), neuromuscular evaluation consisted in four parts: part I, determination of the optimal site for transcranial magnetic stimulation (TMS); part II, determination of the optimal TMS intensity (recruitment curve); part III, assessment of maximal voluntary activation with TMS and nerve electrical stimulation (NES); and part IV, assessment of maximal voluntary activation and muscle contractile properties with muscle electrical stimulation (MES). The isometric elbow flexion fatiguing task consisted in sets of 19 submaximal contractions interspaced by neuromuscular evaluations, until task failure

Electrical nerve and muscle stimulation

Single NES was delivered to the brachial plexus via a cathode electrode (20-mm diameter, Ag–AgCl, Controle Graphique Medical, Brie-Comte-Robert, France) located in the supraclavicular fossa (Erb's point) and an anode, a 5 cm × 5 cm gel pad electrode (Compex SA, Ecublens, Switzerland) on the acromion. NES intensity (64.1 ± 17.5 mA at SL, 58.5 ± 21.2 mA at D1 and 52.6 ± 23.3 mA at D5) corresponded to 130% of the optimal intensity, i.e., the stimulus intensity at which the maximal amplitude of BB M wave was reached. Supramaximal NES was delivered 2 s after the last MVC of the four-contraction sets as single-pulse NES to obtain M-wave (part III of neuromuscular evaluation).

Paired-pulse MES was delivered to the BB via a cathode electrode (20-mm diameter, Ag–AgCl, Controle Graphique Medical) located midway between the anterior edge of the deltoid and the elbow crease. The anode, a 10 cm × 5 cm self-adhesive stimulation electrode (Compex SA), was placed over the bicipital tendon (2–3 cm proximal to the elbow). MES intensity (40.5 ± 15.3 mA at SL, 44.9 ± 11.4 mA at D1 and 41.1 ± 11.3 mA at D5) corresponded to 130% of the optimal intensity, i.e., the stimulus intensity at which the maximal amplitude of doublet force was reached. Supramaximal MES was delivered: (i) during MVCs as paired high-frequency stimuli at 100 Hz (part IV of neuromuscular evaluation) and (ii) 2 s after MVCs in relaxed muscle as paired high- and low-frequency (100 and 10 Hz) stimuli separated by

a 5-s interval (part IV and neuromuscular evaluations during the exercise test).

For both MES and NES, square wave pulses (100-μs duration) were produced via a high-voltage (maximal voltage 400 V) constant-current stimulator (Digitimer DS7, Hertfordshire, UK).

Transcranial magnetic stimulation

A magnetic stimulator (Magstim 200, The Magstim Company, Dyfed, UK) was used to stimulate the motor cortex. Single TMS pulses of 1-ms duration were delivered via a circular coil (135-mm outside diameter) positioned over the vertex of the scalp and held tangentially to the skull. The coil was positioned to preferentially activate the left motor cortex (contralateral to the right arm) and elicit the largest MEP in the BB and the BR with only a small MEP in the TB during isometric elbow flexion at 20% MVC with a stimulus intensity of 70% of maximal stimulator power output (part I of neuromuscular evaluation; Fig. 2a). The optimal stimulus site was defined in each session and marked on a white hypoallergenic tape which was fixed directly to the scalp to ensure reproducibility of the stimulus conditions for each subject throughout the entire session. After 4 min of rest, TMS during brief (3 s) isometric elbow flexions at 40% MVC [i.e., contraction intensity slightly lower than the force level required during the fatiguing task (Jubeau et al. 2017); part II] were performed at 30, 40, 50, 60, 70, 80, 90, and 100% of maximal stimulator power output in random order. Four consecutive contractions were performed at each stimulus intensity with 10 s between contractions at the same stimulation intensity and 30 s between series of four contractions. The stimulus intensity (67.3 ± 12.7% at SL, 66.4 ± 6.7% at D1, and 70.0 ± 8.9% at D5 of stimulator maximal power output) that elicited the largest right BB and BR MEPs with small MEP of the right TB (amplitude <10% of maximal BB M-wave) was considered optimal and employed throughout the protocol, as previously suggested (Todd et al. 2003).

After another 4 min of rest, VA_{TMS} assessment (part III) consisted of three sets of four brief (3 s) contractions at 100% MVC, 75% MVC, and 50% MVC (calculated from the first MVC of each set) and finally 100% MVC (to assess Mmax during NES immediately after MVC) with 10 s of rest between contractions and 30 s between series (Todd et al. 2003). TMS was delivered during the first three contractions. VA_{TMS} during the isometric elbow flexion test was assessed from one set of three brief (3 s) contractions at 100, 75, and 50% MVC (see below). Strong verbal encouragement was given during MVCs and target force levels and real-time visual feedbacks of the force produced were provided to the subject via custom software (Labview 8, National Instrument, Austin, TX) on a computer screen throughout the experiment. The subjects were instructed to return as quickly as possible to the desired force level after each TMS pulse elicited during voluntary contractions to allow valid assessment of CSP (Mathis et al. 1998).

Electromyographic recordings

The EMG signals of the right BB, BR, and TB were recorded using bipolar silver chloride surface electrodes of 20-mm diameter (Contrôle Graphique Medical) during the voluntary contractions and electrical/magnetic stimuli. The recording electrodes were secured lengthwise to the skin over the muscle belly following SENIAM recommendations (Hermens et al. 2000), with an inter-electrode distance of 25 mm. The positions of the EMG electrodes were marked on the skin with indelible ink on the first experimental session to ensure that they were placed in the same location at subsequent visits. Low impedance ($Z < 5$ kΩ) at the skin–electrode surface was obtained by abrading the skin with fine sand paper and cleaning with alcohol. EMG signals were amplified, band-pass filtered (5 Hz–1 kHz, input impedance 200 MΩ, common mode rejection ratio 85 dB, gain 1000) and recorded at a sampling rate of 2 kHz using BioAmp and PowerLab systems (ADInstruments, Bella Vista, Australia) to be stored on a computer for subsequent analysis with the LabChart 7 software (ADInstrument).

NIRS measurements

Oxy(HbO_2)-, deoxy(HHb)-, and total(HbTot)-haemoglobin concentration changes were estimated throughout testing sessions over multiple sites using a two-wavelength (780 and 850 nm) multichannel, continuous wave NIRS system (Oxymon MkIII, Artinis Medical Systems, The Netherlands). Elbow flexor muscle hemodynamics were assessed from the right BB using a 4-cm interoptode distance. Probe holder was secured to the skin using double-sided tape and covered with a black sweatband to shield the optodes from ambient light. Left pre-frontal cortex hemodynamics were assessed between Fp1 and F3 locations according to the international 10–20 EEG system with 3.5-cm interoptode distance.

The probe holder was secured to the skin with double-sided tape and maintained with Velcro headbands. Data were recorded continuously at 10 Hz and filtered with a 2-s width moving Gaussian smoothing algorithm before analysis.

Cardiorespiratory parameters and RPE

Heart rate, arterial oxygen saturation (SpO_2) by pulse oximetry at the ear lobe, and end-tidal CO_2 partial pressure

($PetCO_2$) from a cannula connected to a face-mask were measured continuously (Mindray, iPM9800, China). The subjects were asked to report their rate of perceived exertion (RPE), i.e., how hard they perceived the exercise, at the end of each 19-contractions set and at task failure, using a 100-mm visual analog scale, with ''no effort'' on one end (0 mm) and ''maximal effort'' on the other (100 mm).

Symptoms of acute mountain sickness

Subjects were asked the morning of every experimental days to complete self-reported questionnaires for acute mountain sickness evaluation according to the Lake Louise Score (five items; Roach et al. 1993) and the cerebral subscore of the Environmental Symptom Questionnaire (11 items; Sampson et al. 1983) The presence of acute mountain sickness was defined as Lake Louise Score $\geq$3 and cerebral subscore of the Environmental Symptom Questionnaire $\geq$0.7.

Data analysis

The torque amplitudes of paired pulses at 100 and 10 Hz (Db100 and Db10, respectively) MES were determined. The presence of low-frequency fatigue during and after the fatiguing task was evaluated from the change in the ratio of Db10 to Db100 (Db10:100) (Verges et al. 2009). Before and after the fatiguing task, Mmax peak-to-peak amplitude in relaxed muscles was measured from single-pulse NES. VA_{MES} was assessed by twitch interpolation using the superimposed and potentiated doublet amplitudes elicited by 100-Hz paired-pulse MES during and after MVC and calculated from the equation: $VA_{MES} = [1 - (\text{superimposed 100-Hz MES paired pulse amplitude} \times Db100^{-1})] \times 100$.

MEP peak-to-peak amplitudes of elbow flexor muscles during TMS superimposed on submaximal and maximal contractions were normalized to Mmax peak-to-peak amplitude. The duration of the CSP was determined visually and defined as the duration from the stimulus to the return of continuous voluntary EMG (Gruet et al. 2014; Sidhu et al. 2009). VA_{TMS} was quantified by measurement of the force responses to TMS. Because motor cortex and spinal cord excitability increase during voluntary contractions, it was necessary to estimate rather than directly measure the amplitude of the resting twitch evoked by motor-cortex TMS (Goodall et al. 2010; Todd et al. 2003). The mean superimposed twitch (SIT) amplitude evoked during contractions at 100, 75, and 50% MVC was calculated, and the *y*-intercept of the linear regression between the SITs and voluntary force was used to quantify the estimated resting twitch (ERT). When linear regressions were not linear ($R^2 < 0.9$), ERT was excluded and VA_{TMS} was not calculated for the considered set of contractions (Hunter et al. 2006). ERT was linear for all subjects for at least one set both before the fatiguing task and at task failure, permitting VA_{TMS} to be determined in all subjects. Cortical VA (%) was then calculated using the equation: $VA_{TMS} = [1 - (SIT \times ERT^{-1})] \times 100$. During the fatiguing task, when the linear regression was <0.9 (in 7% of the measurements), VA_{TMS} was extrapolated as the average value between VA_{TMS} values measured at the previous and next assessment timepoints. Peak forces measured during stimulations, MEPs, CSPs, M-waves, and VA before and after the fatiguing task were calculated as the averaged values obtained during the three sets of contractions.

HbO_2, HHb, and HbTot concentrations are delta from the baseline value before the start of the fatiguing task for each experimental session.

Data from the three experimental sessions were compared over four timepoints: (i) before the fatiguing task; (ii) at 50% and (iii) 100% of the duration of the shortest fatiguing task for a given subject (i.e., to allow comparison of the three sessions at isotime); and (iv) at task failure. If no neuromuscular evaluation corresponded to exactly 50 or 100% of the duration of the shortest fatiguing task for a given subject, the nearest neuromuscular evaluation was considered.

Statistical analysis

All statistical procedures were completed on Statistica version 10 (Statsoft, Tulsa, OK). Normality of distribution and homogeneity of variances of the main variables were confirmed using a Shapiro–Wilk normality test and the Levene's test, respectively. Two-way ANOVA (condition $\times$ time) with repeated measures were performed for each dependent variable. Post-hoc Tukey's tests were applied to determine a difference between two mean values if the ANOVA revealed a significant main effect or interaction effect. For all statistical analyses, a two-tailed alpha level of 0.05 was used as the cutoff for significance. All data are presented as mean values $\pm$ SD.

Results

Exercise performance

Exercise duration to task failure was not significantly different between the three experimental conditions (SL 1095 $\pm$ 562 s; D1: 1132 $\pm$ 516 s; D5: 1440 $\pm$ 689 s; $F = 2.7$, $p = 0.098$).

Cardiorespiratory parameters and RPE

Changes in SpO_2, heart rate, $PetCO_2$, and RPE during the fatiguing task in each experimental condition are shown in Table 1. SpO_2 was significantly lower throughout the fatiguing task at D1 (mean value 91 ± 5%) and D5 (91 ± 5%) compared to SL (99 ± 2%, main effect of condition, $F = 38.1$, $p < 0.001$), without significant difference between D1 and D5. SpO_2 increased significantly from before exercise to task failure at D1 and D5 but not at SL (condition × time interaction, $F = 4.5$, $p < 0.001$). Heart rate was significantly higher at D1 (mean value 104 ± 20 bpm) and D5 (104 ± 22 bpm) compared to SL (93 ± 22 bpm, main effect of condition, $F = 5.4$, $p = 0.013$) throughout the fatiguing task, without significant difference between D1 and D5. $PetCO_2$ was significantly lower throughout the fatiguing task at D1 (mean value 24 ± 5 mmHg) and D5 (23 ± 4 mmHg) compared to SL (34 ± 6 mmHg, main effect of condition, $F = 70.1$, $p < 0.001$), without significant difference between D1 and D5. RPE was not significantly different between the three experimental conditions.

Table 1 Cardiorespiratory parameters and rating of perceived exertion before and during the fatiguing task at sea level (SL) and after 1 (D1) and 5 (D5) days of altitude exposure

	PRE	50%	100%	TF	ANOVA main condition effect
SpO_2 (%)					
SL	98 ± 1	98 ± 1	99 ± 1	99 ± 1	
D1	86 ± 4	92 ± 5	93 ± 5	93 ± 4	<0.001
D5	88 ± 3	90 ± 5	92 ± 5	92 ± 6	
HR (bpm)					
SL	71 ± 17	93 ± 18	103 ± 17	105 ± 18	
D1	84 ± 15	104 ± 14	114 ± 17	116 ± 18	0.009
D5	82 ± 17	102 ± 14	111 ± 18	120 ± 18	
$PetCO_2$ (mmHg)					
SL	38 ± 4	35 ± 5	32 ± 5	30 ± 7	
D1	29 ± 2	24 ± 4	21 ± 5	21 ± 4	<0.001
D5	27 ± 3	24 ± 3	21 ± 3	20 ± 4	
RPE (0–100 mm)					
SL	16 ± 15	81 ± 19	95 ± 7	97 ± 5	
D1	15 ± 15	84 ± 15	96 ± 7	97 ± 5	0.092
D5	9 ± 9	74 ± 19	91 ± 10	97 ± 4	

Mean values ± SD; $n = 11$

ANOVA revealed a significant time effect for all variables ($p < 0.05$)

PRE before the fatiguing task, *50%* 50% of the duration of the shortest test, *100%* 100% of the duration of the shortest test, *TF* task failure, *SpO_2* arterial oxygen saturation, *HR* heart rate, *$PetCO_2$* end-tidal carbon dioxide partial pressure, *RPE* rating of perceived exertion

Symptoms of acute mountain sickness

Symptoms were significantly increased at D1 (Lake Louise score 5 ± 3; cerebral subscore of the Environmental Symptom Questionnaire 0.80 ± 0.85) compared to SL (1 ± 1; 0.01 ± 0.12) and D5 (1 ± 1; 0.07 ± 0.10), with no difference between SL and D5. Seven subjects presented acute mountain sickness at D1 and none at D5.

Neuromuscular fatigue

MVC, VA, and evoked elbow flexor force as well as BB, BR and TB Mmax before the fatiguing task did not differ between experimental conditions (data not shown, all $p > 0.05$). Changes in maximal and evoked elbow flexor force during the fatiguing task are provided in Fig. 3. MVC changes were not significantly different between the three experimental conditions (condition × time interaction, $F = 0.7$, $p = 0.649$). The reduction in Db100 at task failure (condition × time interaction, $F = 3.0$, $p = 0.011$) was significantly larger at D5 compared to SL. Db10:100 was not significantly different between the three experimental conditions (condition × time interaction, $F = 0.6$, $p = 0.741$). BB and BR Mmax did not change during the fatiguing task and were not significantly different between all experimental conditions (data not shown, all $p > 0.05$).

A significant main effect of experimental condition was observed for VA_{TMS} ($F = 4.0$, $p = 0.034$; Fig. 3d) with lower values at D1 compared to D5. There was, however, no experimental condition × time interaction ($F = 1.7$, $p = 0.139$). VA_{MES} declines were not different between experimental conditions (condition × time interaction, $F = 0.8$, $p = 0.466$; Fig. 3e).

Corticospinal excitability and inhibition

BB and BR MEP.$Mmax^{-1}$ and CSP during the recruitment curve are shown in Fig. 4. BB MEP.$Mmax^{-1}$ (Fig. 4a) did not differ between the three experimental conditions. BR MEP.$Mmax^{-1}$ (Fig. 4b) showed greater values at 90 and 100% of maximal stimulator power output at SL compared to D5 (experimental condition × time interaction; $F = 2.2$, $p = 0.010$). A main effect of experimental condition was observed for BB (Fig. 4c) and BR (Fig. 4d) CSP with greater values at D1 and D5 compared to SL ($F = 5.5$, $p = 0.012$; $F = 5.3$, $p = 0.014$ for BB and BR, respectively).

MEP.$Mmax^{-1}$ and CSP during the fatiguing task are shown in Table 2. A main effect of experimental condition was observed for BR MEP.$Mmax^{-1}$ at 50% MVC ($F = 4.6$, $p = 0.022$), with lower values at D5 compared to SL. BB MEP.$Mmax^{-1}$ at 50% MVC also tended to be reduced at D5 (main effect of experimental condition $F = 3.4$, $p = 0.055$). There was no significant BR

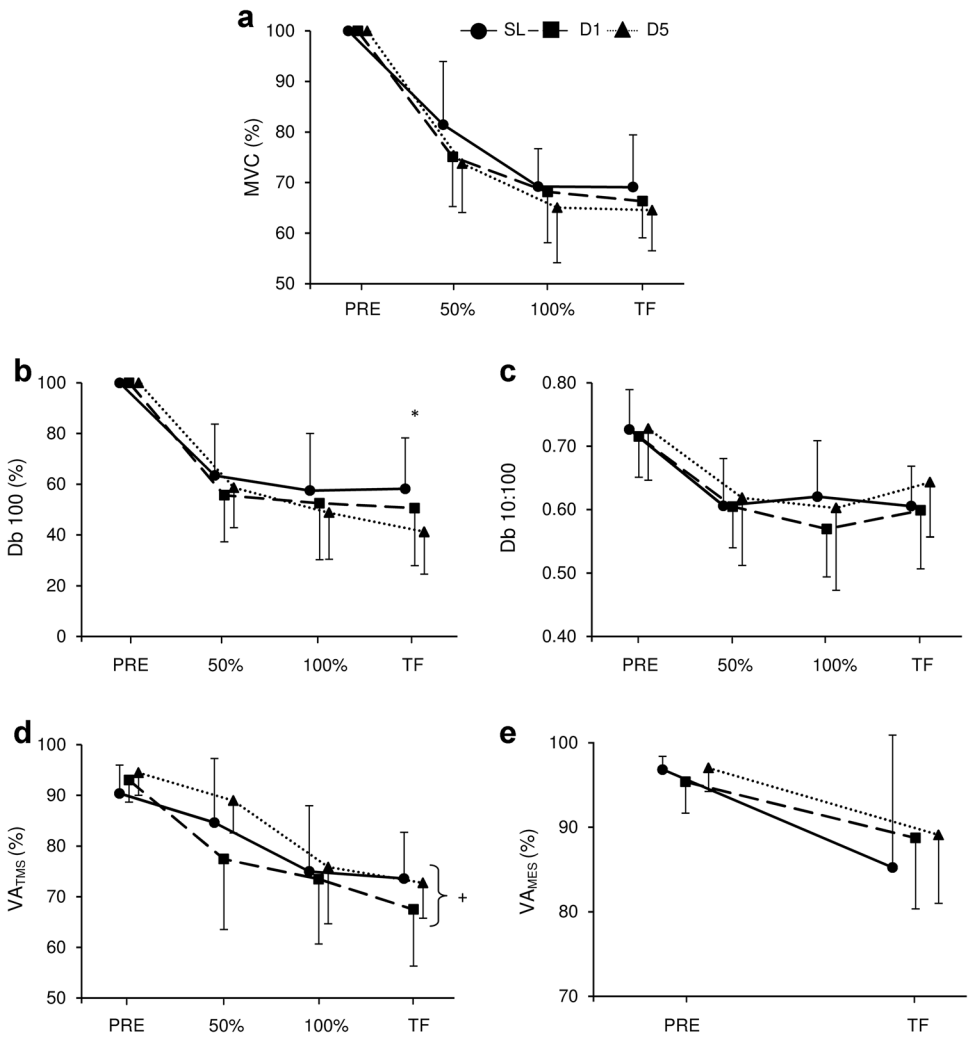

Fig. 3 Maximal voluntary contraction force (MVC, **a**), paired-pulse peak force at 100 Hz (DB 100, **b**), ratio of paired-pulse peak force at 10 and 100 Hz (Db 10:100, **c**) and maximal voluntary activation level of the arm flexor muscles assessed by transcranial magnetic stimulation (VA_{TMS}, **d**), and electrical muscle stimulation (MES, **e**) during the isometric arm flexion test at sea level (SL), after 1 (D1) and 5 (D5) days of altitude exposure. *PRE* before exercise, *50%* 50% of the duration of the shortest test, *100%* 100% of the duration of the shortest test, *TF* after task failure. *Asterisk* significant difference between SL and D5. *Plus sign* significant difference between D1 and D5 (main condition effect). Values at each timepoint are slightly shifted to avoid *error bars overlap*

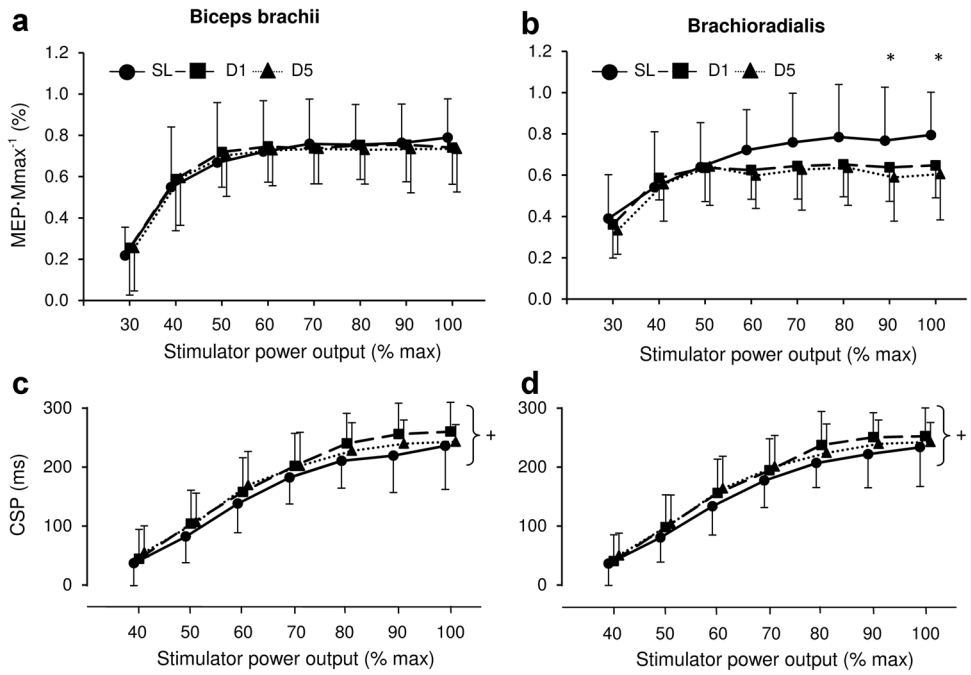

Fig. 4 Biceps brachii and brachioradialis motor evoked potential normalized to M-wave amplitude ($MEP \cdot Mmax^{-1}$, **a**, **b**) and cortical silent period duration (CSP, **c**, **d**) assessed by transcranial magnetic stimulation (TMS) at increasing intensity levels at sea level (SL) and after 1 (D1) and 5 (D5) days of altitude exposure. *Asterisk* significant difference between SL and D5. *Plus sign* significant difference between D1 and D5 compared to SL (main condition effect). CSP at 30% of maximal stimulator power output were not detectable in all subjects. Values at each timepoint are slightly shifted to avoid *error bars overlap*

Table 2 Corticospinal excitability and inhibition, before, during, and after the fatiguing task at sea level (SL) and after 1 (D1) and 5 (D5) days of altitude exposure

SL				D1				D5				ANOVA main condition effect
PRE	50%	100%	TF	PRE	50%	100%	TF	PRE	50%	100%	TF	
Biceps brachii												
MEP·Mmax^{-1} (%)												
50% MVC	75 ± 19	80 ± 16	75 ± 12	70 ± 16	76 ± 16	88 ± 28	78 ± 23	68 ± 20	76 ± 19	77 ± 19	66 ± 17	58 ± 22	0.055
75% MVC	73 ± 16	75 ± 18	75 ± 14	71 ± 15	75 ± 14	79 ± 29	74 ± 18	70 ± 14	73 ± 20	75 ± 14	78 ± 12	69 ± 16	0.955
100% MVC	62 ± 12	64 ± 12	64 ± 18	62 ± 16	62 ± 11	74 ± 23	61 ± 15	63 ± 13	58 ± 13	69 ± 13	66 ± 16	61 ± 14	0.708
CSP (ms)													
50% MVC	167 ± 60	173 ± 61	171 ± 54	168 ± 55	182 ± 61	200 ± 53	189 ± 55	187 ± 57	197 ± 62	206 ± 55	207 ± 55	200 ± 55	0.067
75% MVC	164 ± 40	163 ± 56	173 ± 66	167 ± 60	189 ± 59	189 ± 51	189 ± 50	186 ± 57	200 ± 63	204 ± 55	202 ± 50	202 ± 60	0.015
100% MVC	167 ± 68	177 ± 63	178 ± 62	174 ± 61	204 ± 66	214 ± 59	209 ± 61	203 ± 61	215 ± 73	215 ± 55	224 ± 60	217 ± 64	0.003
Brachioradialis													
MEP·Mmax^{-1} (%)													
50% MVC	68 ± 17	86 ± 29	70 ± 27	62 ± 20	63 ± 15	78 ± 34	57 ± 18	51 ± 11	59 ± 18	70 ± 31	50 ± 20	46 ± 17	0.022
75% MVC	63 ± 19	47 ± 17	58 ± 17	58 ± 20	53 ± 9	66 ± 41	49 ± 13	50 ± 9	49 ± 12	58 ± 23	54 ± 25	48 ± 9	0.802
100% MVC	49 ± 13	50 ± 23	51 ± 29	46 ± 15	41 ± 7	50 ± 15	42 ± 18	39 ± 8	37 ± 8	36 ± 12	45 ± 15	42 ± 9	0.105
CSP (ms)													
50% MVC	162 ± 56	175 ± 60	173 ± 56	170 ± 54	181 ± 59	201 ± 54	187 ± 57	187 ± 57	192 ± 63	203 ± 55	211 ± 54	198 ± 57	0.006
75% MVC	164 ± 57	175 ± 60	175 ± 67	166 ± 61	183 ± 61	194 ± 54	189 ± 52	189 ± 61	194 ± 63	212 ± 61	207 ± 54	199 ± 59	0.101
100% MVC	171 ± 70	180 ± 64	188 ± 76	174 ± 62	202 ± 64	218 ± 60	208 ± 60	203 ± 62	213 ± 71	221 ± 63	232 ± 66	218 ± 64	0.010
Triceps brachii													
MEP·Mmax^{-1} (%)													
50% MVC	13 ± 13	10 ± 7	9 ± 6	9 ± 6	10 ± 9	13 ± 11	12 ± 9	14 ± 12	11 ± 7	9 ± 6	12 ± 9	14 ± 13	0.646
75% MVC	17 ± 14	22 ± 27	19 ± 17	17 ± 12	10 ± 8	14 ± 10	15 ± 14	17 ± 15	13 ± 14	11 ± 10	13 ± 10	15 ± 13	0.438
100% MVC	19 ± 14	19 ± 15	23 ± 26	24 ± 19	20 ± 16	23 ± 13	24 ± 17	30 ± 23	26 ± 23	18 ± 14	22 ± 17	31 ± 23	0.651

Mean values ± SD; $n = 11$

Triceps brachii MEP·Mmax^{-1} are provided to show that by optimizing the site of transcranial magnetic stimulation for elbow flexors, this antagonist muscle was minimally recruited

PRE before the fatiguing task, *50%* 50% of the duration of the shortest test, *100%* 100% of the duration of the shortest test, *TF* after task failure, *MEP* motor evoked potential, *CSP* cortical silent period, *Mmax* maximal M-wave

and BB MEP.Mmax^{-1} difference at 75 and 100% MVC between experimental conditions as well as for TB MEP.Mmax^{-1} at any contraction intensity (all $p > 0.05$).

A main effect of experimental condition was observed for BB CSP at 75% ($F = 5.2$, $p = 0.015$) and 100% ($F = 7.8$, $p = 0.003$) MVC, with shorter values at SL compared to D5 at 75% MVC and compared to D1 and D5 at 100% MVC (Table 2). A main effect of experimental condition and an experimental condition × time interaction were observed for BR CSP at 50% ($F = 6.9$, $p = 0.006$ and $F = 2.3$, $p = 0.049$, respectively) and 100% ($F = 2.7$, $p = 0.021$ and $F = 2.7$, $p = 0.021$, respectively) MVC, with shorter values throughout the fatiguing task at SL compared to D1 and D5.

Pre-frontal cortex and biceps brachii oxygenation

Changes in pre-frontal and BB HbO_2, HHb, and HbTot concentrations during the fatiguing task in each experimental condition are shown in Fig. 5. Pre-frontal cortex HHb (main effect of condition, $F = 6.3$, $p = 0.008$; condition × time interaction, $F = 2.8$, $p = 0.017$) was higher at SL compared to D1 throughout the fatiguing task and compared to D5 at 100% of the shortest test duration only. Pre-frontal cortex HbO_2, HbTot and BB HbO_2, HHb, and HbTot did not differ between experimental conditions (all $p > 0.05$).

Discussion

This is the first study to evaluate the effects of several days at high altitude on neuromuscular fatigue development and corticospinal excitability and inhibition during an isolated muscle exercise using neurostimulation and TMS. The results indicate that high-altitude exposure did not elicit a larger amount of central fatigue during intermittent isometric elbow flexions performed to exhaustion, while it

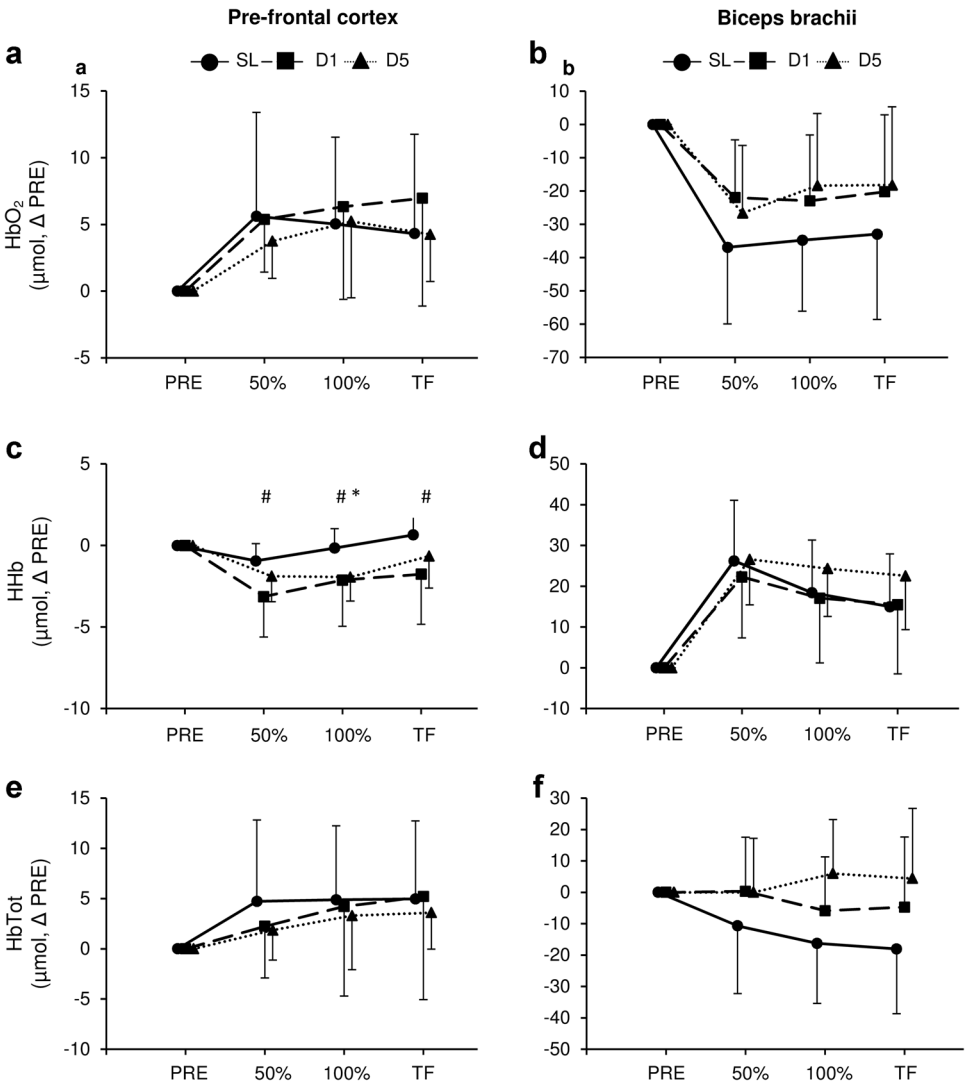

Fig. 5 Pre-frontal and biceps brachii oxyhaemoglobin (HbO_2, **a**, **b**), deoxyhaemoglobin (HHb, **c**, **d**), and total haemoglobin (HbTot, **e**, **f**) assessed by near-infrared spectroscopy during the isometric arm flexion test at sea level (SL) and after 1 (D1) and 5 (D5) days of altitude exposure. *PRE* before the fatiguing task; *50%* 50% of the duration of the shortest test, *100%* 100% of the duration of the shortest test, *TF* after task failure. *Asterisk* significant difference between SL and D1; *plus sign* significant difference between SL and D5. Values at each timepoint are slightly shifted to avoid *error bars overlap*

induced a decrease in corticospinal excitability and an increase in corticospinal inhibition compared to SL. These results do not confirm that performance during this type of exercise may be limited by central fatigue at high-altitude (at least at 4350 m), in spite of reduced corticospinal excitability and enhanced inhibition.

Fatigue development at high altitude

The main aim of our study was to determine the effect of short-term high-altitude exposure and acclimatization over 5 days on the development of exercise-induced central fatigue. We hypothesized that short-term high-altitude exposure (D1) would elicit a larger amount of supraspinal fatigue during exercise and that acclimatization (D5 when symptoms of acute mountain sickness have disappeared) would normalize these changes. Acute severe hypoxic exposure (<1 h, FiO_2 0.13–0.10, equivalent to ~3500–5000 m) has been shown to accentuate VA_{TMS} reduction during cycling (Goodall et al. 2012, 2014) and leg extensions (Goodall et al. 2010). During repeated isometric leg extensions, Goodall et al. (2010) showed that acute severe (FiO_2 0.10) but not mild and moderate (FiO_2 0.16–0.13) normobaric hypoxia accentuates VA_{TMS} reduction (from 94.5 ± 4.7 to 70.7 ± 24.3%) compared to normoxia (reduction from 94.8 ± 4.8 to 86.8 ± 10.9%). In acute severe hypoxia, SpO_2 before exercise was 74.2 ± 6.6%, while at the end of exercise, it was 78.7 ± 11.8%. In the present study, VA_{TMS} was reduced from 90.4 ± 5.6 and 93.0 ± 4.3% before exercise to 73.6 ± 9.1 and 67.5 ± 11.2% at exhaustion at SL and after 24 h at 4350 m, respectively. While in both cases elbow flexions to exhaustion induced large amount of supraspinal fatigue, VA_{TMS} reduction did not differ between conditions. SpO_2 levels at D1 were 86 ± 4% before exercise and 93 ± 4% at exhaustion. These levels of hypoxemia were similar to the mild or moderate hypoxic conditions in the study of Goodall et al. (2010), where similar leg extension-induced supraspinal fatigue was observed compared to normoxia.

While cerebral tissue oxygenation index at rest was significantly reduced under the present high altitude conditions compared to SL as previously reported (Rupp et al. 2014), the present results show similar kinetics of pre-frontal HbO_2 and HbTot during isolated exercise and larger decrease in HHb at high altitude compared to SL. Even though an increase in HbO_2 and a reduction in HHb represent the typical NIRS response associated with neurovascular coupling, the greater reduction in HHb during exercise at high altitude compared to SL suggests a larger neurovascular coupling which may contribute to preserved central drive. Other potential mechanisms leading to impaired central drive under hypoxic conditions are the inhibitory afferences originating from fatigued peripheral muscles (Amann et al. 2009). In the present study, peripheral fatigue both at isotime and at exhaustion was not accentuated at D1 compared to SL (as suggested by similar reductions in Db100 and Db10:100), which suggests that inhibitory afferences were similar under both conditions. Hence, both the preserved kinetics of cerebral oxygenation and the similar amount of peripheral fatigue during elbow flexions may account for the absence of difference in supraspinal fatigue between SL and D1.

Only one study has assessed VA_{TMS} and exercise-induced supraspinal fatigue following several days at high altitude (Goodall et al. 2014). In this study, the authors found that acute severe hypoxia (FiO_2 0.105) accentuated VA_{TMS} reduction after cycling at 50% of maximal normoxic power output for 10.1 ± 1.4 min. However, acclimatization for 14 days at 5260 m normalized post-cycling VA_{TMS} to SL values. This normalization of supraspinal fatigue with acclimatization may be due to the large improvement in SpO_2 (~15%) and cerebral O_2 delivery (~20%) observed after 2 weeks of altitude exposure compared to acute hypoxic exposure (Goodall et al. 2014). In the present study, a significant main effect of experimental condition was observed for VA_{TMS} indicating higher values at D5 compared to D1, without interaction between the experimental condition and exercise time. These results suggest an enhanced central drive after five compared to 1 day of high altitude exposure but similar development of supraspinal fatigue during isolated muscle exercise. This increased central drive at D5 was observed despite similar SpO_2 and pre-frontal oxygenation levels before and during exercise compared to D1. Hence, mechanisms other than increased cerebral oxygen delivery may explain the higher VA_{TMS} values observed after initial acclimatization experienced following 5 days at high altitude. One could suggest that a training effect may account for the larger VA_{TMS} values observed at D5, i.e., when subjects performed the elbow flexion tests for the third time within less than 3 weeks (see "Limitations"). Greater central drive and slightly longer exercise duration (although not significantly) after high-altitude acclimatization may have contributed to the greater amount of peripheral fatigue observed at exhaustion at D5. Despite similar low-frequency fatigue (i.e., Db10:100) in both high altitude and SL conditions (Fig. 3), the larger peripheral fatigue is evidenced by the larger reduction in Db100 at D5. Altogether, the present study demonstrates that both 1 and 5 days at high altitude (4350 m) have no significant effect on VA_{TMS} reduction during elbow flexions, probably because of the low muscle mass involved during this type of exercise and the relatively modest levels of hypoxemia.

Corticospinal excitability at high altitude

While most (Goodall et al. 2010, 2012, 2014; Millet et al. 2012; Rupp et al. 2012) but not all (Szubski et al. 2006) previous studies having investigated the effect of acute (<1 h) hypoxic exposure on corticospinal excitability and inhibition reported no significant alteration at rest, some studies (Goodall et al. 2014; Miscio et al. 2009; Rupp et al. 2012) suggested that longer hypoxic exposure (from 3 h to 14 days) may lead to significant changes. Differences between studies regarding the effect of hypoxia on corticospinal excitability and inhibition may also arise from methodological differences, i.e., TMS performed on relaxed versus contracted muscles or various parameters to assess corticospinal excitability and inhibition (e.g., resting or active motor threshold versus MEP, CSP versus inhibition investigated from paired-pulse TMS).

The effect of hypoxia may differ between unfatigued condition (before exercise) and during exercise when significant alterations in corticospinal excitability and central fatigue occur (Bachasson et al. 2016; Jubeau et al. 2014; Rupp et al. 2015). The present results indicate that (i) both 1 and 5 days of high-altitude exposure reduce corticospinal excitability and increase corticospinal inhibition as suggested by reduced MEP·Mmax^{-1} ($p < 0.05$ for the BR and $p = 0.055$ for the BB at 50% MVC) and increased CSP (Table 2; Fig. 4), (ii) the effect of altitude exposure depends on the force level when TMS is applied, with significant reduction in MEP·Mmax^{-1} at force levels ≤50% MVC (50% MVC before and during exercise and 40% MVC during the recruitment curve), (iii) the effect of altitude exposure is similarly observed before, during and after exercise, and (iv) changes in corticospinal excitability and inhibition occur at high altitude despite similar amount of supraspinal fatigue between SL, D1, and D5.

The investigation of Goodall et al. (2014) is the only one which reported MEP·Mmax^{-1} after several days at high-altitude and suggests an increased corticospinal excitability compared to normoxia. Although the reasons for this contrasting result compared to the present study is unclear (e.g., altitude level, exposure duration, and muscle group), it should be noted that peripheral excitability (Mmax) was significantly modified after 14 days at high altitude (while it was unchanged in the present study) and that similar to the present study, the effect of prolonged high-altitude exposure was observed mostly at moderate (50% MVC) but not high (100% MVC) force levels. At high force levels, the large neuronal output associated with central drive may conceal the effect of hypoxia per se on corticospinal excitability as assessed by MEP·Mmax^{-1}. The fact that significant reductions in MEP·Mmax^{-1} were observed for the BR, while a tendency for lower BB MEP·Mmax^{-1} was observed at 50% MVC also suggests differences between muscle groups. Although the BB is the main elbow flexor, it should be emphasized that the BR shows its largest activation during elbow flexion at high force level (Boland et al. 2008) and, therefore, contributed significantly to the fatiguing task in the present study.

In accordance with the present results, Goodall et al. (2014) reported hypoxia-induced changes in MEP·Mmax^{-1} which were similar both before and after exercise. These authors suggested that the increased corticospinal excitability that they observed after 14 days at high altitude may have contributed to the normalization (compared to SL) of exercise-induced VA_{TMS} reduction. The present study rather suggests a dissociation between changes in corticospinal excitability and inhibition, since VA_{TMS} was reduced to a similar extent at SL, D1, and D5 despite significant changes in MEP·Mmax^{-1} and CSP. This is in accordance with previous studies suggesting that changes in corticospinal excitability and inhibition occurring during exercise can be dissociated from the impairment of voluntary activation (Gandevia et al. 1996; Jubeau et al. 2014; Rupp et al. 2015).

Both Goodall et al. (2014) and Miscio et al. (2009) reported no significant effect of several days at high altitude on CSP which again contrasts with the prolonged CSP duration observed at D1 and D5 in the present study. The increase in CSP was observed both before and during exercise as altitude-induced changes in MEP·Mmax^{-1}. In addition, the increase was observed at all force levels from 50 to 100% MVC. Animal and in vitro studies have shown hypoxia-induced alterations in neurotransmitters and neuronal activities which may underlie the present changes in corticospinal excitability and inhibition at high altitude (Gibson and Duffy 1981; Hansen 1985; Neubauer and Sunderram 2004; Olson et al. 1983). Cummins et al. (1993) showed, for instance, that acute hypoxia induced a major decrease in human cortical neurons excitability due to the modulation (inactivation) of Na^+ channels. These mechanisms may underlie the present changes in corticospinal excitability and inhibition at high altitude. In addition, clinical studies indicate that patients experiencing chronic hypoxia such as patients with obstructive sleep apnea have reduced corticospinal excitability (Joo et al. 2010; Lanza et al. 2015) and enhanced inhibition (Grippo et al. 2005; Joo et al. 2010). Hence, the exposure in the present study for several days to hypoxia, both sustained diurnal hypoxia and probably intermittent nocturnal hypoxia (due to altitude-induced Cheyne–Stokes breathing; Nussbaumer-Ochsner et al. 2012), may induce changes similar to hypoxemic patients, although other mechanisms such as PCO_2 levels may account in disease conditions.

Limitations

To account for a potential training effect associated with three maximal elbow flexion tests performed within 3 weeks, a control group in normoxia would have been needed. Due to the already demanding logistics associated with the present study, including a control group was not possible. It is, however, unlikely that a potential training effect would have significantly influenced the main conclusions of the present study, since (i) MVC force levels did not differ between all three conditions, (ii) one maximal elbow flexion test at SL is unlikely to have significantly reduced the development of supraspinal fatigue (and compensated the deleterious effect of hypoxia) 10 days later at D1, and (iii) a training effect at D5 should have accentuated the reduction in exercise-induced supraspinal fatigue from D1 which we hypothesized due to acclimatization, but no difference in supraspinal fatigue between D1 and D5 was observed. To better characterize the effect of prolonged hypoxic exposure on corticospinal excitability and inhibition, further evaluations are needed such as paired-pulse TMS (to assess inhibitory and facilitatory mechanisms) and cervicomedullary stimulations (to assess spinal mechanisms). At last, while prolonged altitude exposure refers in the present study to 5 days at 4350 m, further studies should provide more insights into the corticospinal adaptations to chronic (several weeks) high altitude exposure.

In conclusion, 1 and 5 days of high-altitude exposure resulted in a similar development of supraspinal fatigue compared to sea level during exhaustive isolated muscle exercise. The involvement of a small muscle mass and the relatively moderate hypoxemia during exercise may be responsible for this result. Interestingly, despite similar levels of supraspinal fatigue, high altitude exposure induced significant changes in corticospinal excitability and inhibition. The functional consequences (e.g., motor control and cognitive performances) of impaired corticospinal excitability and increased corticospinal inhibition at high altitude remain to be elucidated.

Acknowledgements We thank all the subjects for participating in the study.

Compliance with ethical standards

Grants This work has been funded by the Rhone-Alpes Region and the Fond de dotation AGIR pour les maladies chroniques.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

Amann M, Romer LM, Subudhi AW, Pegelow DF, Dempsey JA (2007) Severity of arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise performance in healthy humans. J Physiol 581:389–403

Amann M, Proctor LT, Sebranek JJ, Pegelow DF, Dempsey JA (2009) Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. J Physiol 587:271–283

Amann M, Goodall S, Twomey R, Subudhi AW, Lovering AT, Roach RC (2013) AltitudeOmics: on the consequences of high-altitude acclimatization for the development of fatigue during locomotor exercise in humans. J Appl Physiol (1985) 115:634–642

Bachasson D, Temesi J, Gruet M, Yokoyama K, Rupp T, Millet GY, Verges S (2016) Transcranial magnetic stimulation intensity affects exercise-induced changes in corticomotoneuronal excitability and inhibition and voluntary activation. Neuroscience 314:125–133

Boland MR, Spigelman T, Uhl TL (2008) The function of brachioradialis. J Hand Surg Am 33:1853–1859

Calbet JA, Radegran G, Boushel R, Saltin B (2009) On the mechanisms that limit oxygen uptake during exercise in acute and chronic hypoxia: role of muscle mass. J Physiol 587:477–490

Cummins TR, Jiang C, Haddad GG (1993) Human neocortical excitability is decreased during anoxia via sodium channel modulation. J Clin Invest 91:608–615

Gandevia SC (2001) Spinal and supraspinal factors in human muscle fatigue. Physiol Rev 81:1725–1789

Gandevia SC, Allen GM, Butler JE, Taylor JL (1996) Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. J Physiol 490(Pt 2):529–536

Gibson GE, Duffy TE (1981) Impaired synthesis of acetylcholine by mild hypoxic hypoxia or nitrous oxide. J Neurochem 36:28–33

Goodall S, Ross EZ, Romer LM (2010) Effect of graded hypoxia on supraspinal contributions to fatigue with unilateral knee-extensor contractions. J Appl Physiol 109:10

Goodall S, Gonzalez-Alonso J, Ali L, Ross EZ, Romer LM (2012) Supraspinal fatigue after normoxic and hypoxic exercise in humans. J Physiol 590:2767–2782

Goodall S, Twomey R, Amann M, Ross EZ, Lovering AT, Romer LM, Subudhi AW, Roach RC (2014) AltitudeOmics: exercise-induced supraspinal fatigue is attenuated in healthy humans after acclimatization to high altitude. Acta Physiol (Oxf) 210:875–888

Grippo A, Carrai R, Romagnoli I, Lanini B, Bianchi R, Gigliotti F, Scano G (2005) Cortical excitability in obstructive sleep apnea syndrome: transcranial magnetic stimulation study. Sleep 28:1547–1553

Gruet M, Temesi J, Rupp T, Levy P, Verges S, Millet GY (2014) Dynamics of corticospinal changes during and after high-intensity quadriceps exercise. Exp Physiol 99:1053–1064

Hansen AJ (1985) Effect of anoxia on ion distribution in the brain. Physiol Rev 65:101–148

Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G (2000) Development of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol 10:361–374

Hunter SK, Butler JE, Todd G, Gandevia SC, Taylor JL (2006) Supraspinal fatigue does not explain the sex difference in muscle fatigue of maximal contractions. J Appl Physiol (1985) 101:1036–1044

Joo EY, Kim HJ, Lim YH, Koo DL, Hong SB (2010) Altered cortical excitability in patients with untreated obstructive sleep apnea syndrome. Sleep Med 11:857–861

Jubeau M, Rupp T, Perrey S, Temesi J, Wuyam B, Levy P, Verges S, Millet GY (2014) Changes in voluntary activation assessed by transcranial magnetic stimulation during prolonged cycling exercise. PLoS One 9:e89157

Jubeau M, Rupp T, Temesi J, Perrey S, Wuyam B, Millet GY, Verges S (2017) Neuromuscular fatigue during prolonged exercise in hypoxia. Med Sci Sports Exerc 49(3):430–439

Kayser B, Narici M, Binzoni T, Grassi B, Cerretelli P (1994) Fatigue and exhaustion in chronic hypobaric hypoxia: influence of exercising muscle mass. J Appl Physiol 76:634–640

Lanza G, Lanuzza B, Arico D, Cantone M, Cosentino FI, Pennisi M, Bella R, Pennisi G, Ferri R (2015) Direct comparison of cortical excitability to transcranial magnetic stimulation in obstructive sleep apnea syndrome and restless legs syndrome. Sleep Med 16:138–142

Mathis J, de Quervain D, Hess CW (1998) Dependence of the transcranially induced silent period on the 'instruction set' and the individual reaction time. Electroencephalogr Clin Neurophysiol 109:426–435

Millet GY, Muthalib M, Jubeau M, Laursen PB, Nosaka K (2012) Severe hypoxia affects exercise performance independently of afferent feedback and peripheral fatigue. J Appl Physiol (1985) 112:1335–1344

Miscio G, Milano E, Aguilar J, Savia G, Foffani G, Mauro A, Mordillo-Mateos L, Romero-Ganuza J, Oliviero A (2009) Functional involvement of central nervous system at high altitude. Exp Brain Res 194:157–162

Neubauer JA, Sunderram J (2004) Oxygen-sensing neurons in the central nervous system. J Appl Physiol 96:367–374

Nussbaumer-Ochsner Y, Ursprung J, Siebenmann C, Maggiorini M, Bloch KE (2012) Effect of short-term acclimatization to high altitude on sleep and nocturnal breathing. Sleep 35:419–423

Olson EB Jr, Vidruk EH, McCrimmon DR, Dempsey JA (1983) Monoamine neurotransmitter metabolism during acclimatization to hypoxia in rats. Respir Physiol 54:79–96

Roach RC, Bartsch P, Hackett PH, Oelz O (1993) The lake Louise acute mountain sickness scoring system. In: Sutton JR, Coates J, Houston CS (eds) Hypoxia and molecular medicine. Queen City Printers, Burlington, pp 272–274

Rupp T, Jubeau M, Wuyam B, Perrey S, Levy P, Millet GY, Verges S (2012) Time-dependent effect of acute hypoxia on corticospinal excitability in healthy humans. J Neurophysiol 108:1270–1277

Rupp T, Esteve F, Bouzat P, Lundby C, Perrey S, Levy P, Robach P, Verges S (2014) Cerebral hemodynamic and ventilatory responses to hypoxia, hypercapnia, and hypocapnia during 5 days at 4,350 m. J Cereb Blood Flow Metab 34:52–60

Rupp T, Mallouf TL, Perrey S, Wuyam B, Millet GY, Verges S (2015) CO_2 clamping, peripheral and central fatigue during hypoxic knee extensions in men. Med Sci Sports Exerc 47:2513–2524

Sampson JB, Cymerman A, Burse RL, Maher JT, Rock PB (1983) Procedures for the measurement of acute mountain sickness. Aviat Space Environ Med 54:1063–1073

Sidhu SK, Bentley DJ, Carroll TJ (2009) Locomotor exercise induces long-lasting impairments in the capacity of the human motor cortex to voluntarily activate knee extensor muscles. J Appl Physiol 106:556–565

Szubski C, Burtscher M, Loscher WN (2006) The effects of short-term hypoxia on motor cortex excitability and neuromuscular activation. J Appl Physiol 101:1673–1677

Todd G, Taylor JL, Gandevia SC (2003) Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. J Physiol 551:661–671

Verges S, Maffiuletti NA, Kerherve H, Decorte N, Wuyam B, Millet GY (2009) Comparison of electrical and magnetic stimulations to assess quadriceps muscle function. J Appl Physiol 106:701–710

Verges S, Rupp T, Jubeau M, Wuyam B, Esteve F, Levy P, Perrey S, Millet GY (2012) Cerebral perturbations during exercise in hypoxia. Am J Physiol Regul Integr Comp Physiol 302:R903–R916

Villien M, Bouzat P, Rupp T, Robach P, Lamalle L, Tropres I, Esteve F, Krainik A, Levy P, Warnking JM, Verges S (2013) Changes in cerebral blood flow and vasoreactivity to CO(2) measured by Arterial Spin Labeling after 6 days at 4,350 m. Neuroimage 72:272–279