CO_2 Clamping, Peripheral and Central Fatigue during Hypoxic Knee Extensions in Men

THOMAS RUPP[1,2,3], THIBAULT LE ROUX MALLOUF[1,2], STÉPHANE PERREY[4], BERNARD WUYAM[1,2], GUILLAUME Y. MILLET[2,5,6], and SAMUEL VERGES[1,2]

[1]HP2 Laboratory, Université Grenoble Alpes, Grenoble, FRANCE; [2]U1042, INSERM, Grenoble, FRANCE; [3]Laboratoire de Physiologie de l'Exercice, Université Savoie Mont Blanc, Chambéry, FRANCE; [4]Movement To Health, Montpellier-I University, Euromov, Montpellier, FRANCE; [5]Université de Lyon, Saint-Etienne, FRANCE; and [6]Human Performance Laboratory, Faculty of Kinesiology, University of Calgary, Calgary, AB, CANADA

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ABSTRACT

RUPP, T., T. LE ROUX MALLOUF, S. PERREY, B. WUYAM, G. Y. MILLET, and S. VERGES. CO_2 Clamping, Peripheral and Central Fatigue during Hypoxic Knee Extensions in Men. *Med. Sci. Sports Exerc.*, Vol. 47, No. 12, pp. 2513–2524, 2015. **Introduction**: The central nervous system can play a critical role in limiting exercise performance during hypoxic conditions. Hypocapnia, which is associated with hypoxia-induced hyperventilation, may affect cerebral perfusion. We hypothesized that CO_2 clamping during hypoxic isometric knee extensions would improve cerebral oxygenation and reduce central fatigue. **Methods**: Fifteen healthy men (mean ± SD: age, 25 ± 8 yr; body mass, 72 ± 11 kg; height, 179 ± 7 cm) performed intermittent isometric knee extensions at ~50% of maximal voluntary contraction to task failure in normoxia, hypoxia with CO_2 clamping (arterial O_2 saturation, 80% ± 2%; end-tidal CO_2 partial pressure, 40 ± 2 mm Hg), and hypoxia without CO_2 clamping (arterial O_2 saturation, 80% ± 3%). Transcranial magnetic stimulation and femoral nerve electrical stimulation were used to assess central and peripheral determinants of fatigue. Prefrontal cortex and *quadriceps femoris* oxygenation were monitored by multichannel near-infrared spectroscopy. **Results**: Exercise duration was reduced to a similar extent in hypoxia with CO_2 clamping (997 ± 460 s) or hypoxia without CO_2 clamping (929 ± 412 s) compared to normoxia (1473 ± 876 s; $P < 0.001$). Prefrontal cortex and quadriceps oxygenation were increased (+5.3 ± 8.6 and +2.6 ± 3.0 μmol·cm at task failure, respectively; $P < 0.01$) during hypoxia with CO_2 clamping compared to hypoxia without CO_2 clamping. Transcranial magnetic stimulation maximal voluntary activation decreased to a greater extent at task failure in hypoxia without CO_2 clamping (−18% ± 8%) compared to hypoxia with CO_2 clamping (−9% ± 9%; $P < 0.01$) and normoxia (−10% ± 7%; $P < 0.05$). Conversely, exercise-induced peripheral fatigue was larger in hypoxia with CO_2 clamping than in hypoxia without CO_2 clamping (e.g., Db10-to-Db100 ratio of 0.54 ± 0.12 and 0.63 ± 0.11 at task failure, respectively; $P < 0.05$). **Conclusion**: The results demonstrate that CO_2 clamping can alter central and peripheral mechanisms that contribute to neuromuscular fatigue during hypoxic isometric knee extensions in men. Hypocapnia impairs cerebral oxygenation and central drive but exerts a protective effect against fatigability in muscles. **Key Words:** BRAIN, CARBON DIOXIDE, HYPOXIA, NEUROMUSCULAR FATIGUE

Over the past decade, mechanisms within the brain have been proposed as potential contributing factors to hypoxia-induced reduction in exercise performance (2,14,44). Hypoxia during exercise may affect the central nervous system by impairing oxygenation and motor cortex function (3,13,23,27). Changes in cerebral perfusion and oxygenation during hypoxic exercise result from the effects of hypoxemia and hypocapnia associated with hypoxia-induced hyperventilation (22,44). Some studies have evaluated the effects of hypocapnia on cerebral perfusion and oxygenation by testing the hypothesis that CO_2 clamping during hypoxic exercise increases cerebral blood flow and oxygenation and, as a consequence, improves exercise performance (7,8,32,37). These studies confirmed that CO_2 clamping can increase cerebral perfusion and oxygenation during hypoxic exercise; however, in each study, CO_2 clamping did not improve exercise performance, with either no effect (7,8,32) or reduced exercise performance (37).

Although these results suggest that hypocapnia may not be a limiting factor during hypoxic exercise, important limitations must be considered, especially because CO_2 clamping during hypoxic whole-body exercise (i.e., cycling) increases ventilatory response (7,8,37), which results in two main consequences: first, enhanced ventilation increases arterial oxygenation and, as a consequence, the comparison of hypoxic conditions with CO_2 clamping and hypoxic conditions without CO_2 clamping is performed at different levels of arterial blood oxygen saturation (SpO_2) (7,8,37); and, second, enhanced ventilatory response during maximal whole-body exercise in hypoxia with CO_2 clamping may intensify respiratory muscle work and its systemic consequences (6,42), exacerbate adverse

Address for correspondence: Samuel Verges, Ph.D., Laboratoire HP2 (U1042 INSERM), Université Grenoble Alpes, UF Recherche sur l'Exercice, Hôpital Sud, Avenue Kimberley, Echirolles 38 434, France; E-mail: sverges@chu-grenoble.fr.

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respiratory sensations, and consequently impair exercise performance (37). As opposed to whole-body exercise, isolated muscle exercise induces relatively modest cardiorespiratory responses and is mostly limited by neuromuscular mechanisms (5,13). Therefore, this type of exercise appears to be a useful alternative model for assessing the effects of hypoxia-induced hypocapnia on cerebral response and exercise performance.

Previous studies that investigated the effects of CO_2 clamping on prevention of hypocapnia during hypoxic exercise focused on changes in cerebral perfusion and oxygenation (assessed by transcranial Doppler and near-infrared spectroscopy (NIRS)) as potential mechanisms responsible for changes in exercise performance (7,8,32,37). These studies hypothesized that a reduction in cerebral oxygenation due to hypocapnia impairs the ability of the brain to drive muscles (i.e., leading to larger central fatigue) (9) under hypoxic versus normoxic conditions. However, no study to date has investigated the effects of CO_2 clamping during hypoxic exercise on objective peripheral (impairment of excitation–contraction coupling) and central (activation deficit) determinants of fatigue. This specific evaluation is particularly needed because CO_2 clamping may affect central fatigue through its effects on cerebral blood flow but might also influence mechanisms of peripheral muscle fatigue by causing respiratory acidosis (7,37,38). The combination of these central and peripheral effects might explain the observed lack of improvement (7,8,32) or even reduction (37) in maximal exercise performance previously reported in studies of hypoxic CO_2 clamping.

The aim of the present study was to evaluate the effects of CO_2 clamping on peripheral and central determinants of fatigue, as assessed by peripheral nerve electrical stimulation and transcranial magnetic stimulation (TMS), during isometric knee extensions performed in hypoxia. Intermittent isometric knee extensions were performed to task failure (TF) in normoxia and twice in hypoxia at identical SpO_2 levels (80%): once with CO_2 clamping at 40 mm Hg and once without CO_2 manipulation. We hypothesized that CO_2 clamping in hypoxia would i) enhance cerebral oxygenation and reduce central fatigue, ii) increase peripheral muscle fatigue and, consequently, iii) induce no significant change in time to TF compared to hypoxia without CO_2 clamping.

MATERIALS AND METHODS

Subjects. Fifteen healthy, physically active men (mean ± SD: age, 25 ± 8 yr; body mass, 72 ± 11 kg; height, 179 ± 7 cm) were studied. All subjects were nonsmokers and had no history of cardiorespiratory or neuromuscular disease. Subjects refrained from physical exercise 2 d before the tests, abstained from drinking caffeinated beverages on test days, and had their last meal at least 2 h before the tests. The study was approved by the local ethics committee (CPP Sud-Est V, 2010-A00121-38) and was performed according to the Declaration of Helsinki. Subjects were fully informed of the procedure and risks involved and gave their written informed consent.

Study design. After a familiarization session, subjects performed intermittent isometric knee extensions to TF under three experimental conditions: normoxia, hypoxia without CO_2 clamping, and hypoxia with CO_2 clamping. Before, during, and after exercise, neuromuscular evaluations were performed with TMS and femoral nerve electrical stimulation (FNES) to assess voluntary activation (VA), motor cortex excitability, neuromuscular transmission, and muscle contractility. Electromyography (EMG) signals of the *vastus lateralis* (VL), *rectus femoris* (RF), and *biceps femoris* (BF) muscles were measured continuously. In addition, cerebral and muscle oxygenation were monitored continuously during exercise with NIRS.

Familiarization session. During an initial familiarization session, each subject was familiarized with TMS, FNES, and the isometric knee extension ergometer for performing submaximal voluntary contraction and maximal voluntary contraction (MVC). After measurement of MVC, subjects performed isometric knee extensions at 50% of MVC until TF (see later discussion). If the subjects exercised less than 12 min during the familiarization session, the initial target torque during the experimental sessions was set at 45% of MVC (n = 5); if the subjects exercised more than 25 min, the initial target torque was set at 55% of MVC (n = 3); otherwise, 50% of MVC was used as the initial target torque for the experimental sessions (n = 7). This allows reduction of intersubject differences in exercise duration.

Experimental sessions. At least 1 wk after the familiarization session, three experimental sessions (separated by at least 72 h) were performed in random order. Subjects inhaled i) a normoxic gas mixture (inspiratory oxygen fraction (FiO_2) = 0.21; Normoxia); ii) a hypoxic gas mixture that was continuously adjusted (FiO_2 = 0.08–0.13) to maintain SpO_2 at 80% (Hypoxia without CO_2 Clamping); or iii) a hypoxic gas mixture that was individually and continuously adjusted (FiO_2 = 0.08–0.13) to maintain SpO_2 at 80% while end-tidal CO_2 partial pressure ($PetCO_2$) was kept at 40 mm Hg by individually and continuously adjusting inspiratory CO_2 fraction (Hypoxia with CO_2 Clamping).

Subjects breathed through a face mask over all test sessions and were blinded to gas mixture composition delivered by an IsoCap-Altitrainer 200® (SMTEC, Nyon, Switzerland). They laid on a comfortable isometric knee extension ergometer with the right hip angle set at 140° of flexion and with the knee joint angle set at 110° of flexion. The distal part of the right ankle was connected with a noncompliant strap to a strain gauge (Captels, St. Mathieu de Treviers, France) 3–5 cm above the tip of the lateral malleolus. Subjects had their hips and shoulders firmly secured to the bed with noncompliant straps to minimize body movements.

After the initial neuromuscular evaluation, knee extensions consisted of sets of 19 intermittent submaximal isometric contractions (5 s on/3 s off; total set duration, 152 s)

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interspaced by neuromuscular evaluations (lasting 40 s; see later discussion and Fig. 1). Target torque during contractions was initially set at 45%, 50%, or 55% of MVC, depending on performance during the familiarization session, after which it was increased by 5% of MVC every two sets from the fifth set. This progressive increase in target torque was performed to further reduce intersubject differences in exercise duration because preliminary experiments showed very large time to TF in some subjects when target torque remained constant. TF 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 contraction was not at least 4 s in duration or if mean contraction torque was lower than 95% of target torque). Neuromuscular evaluation was repeated immediately at TF.

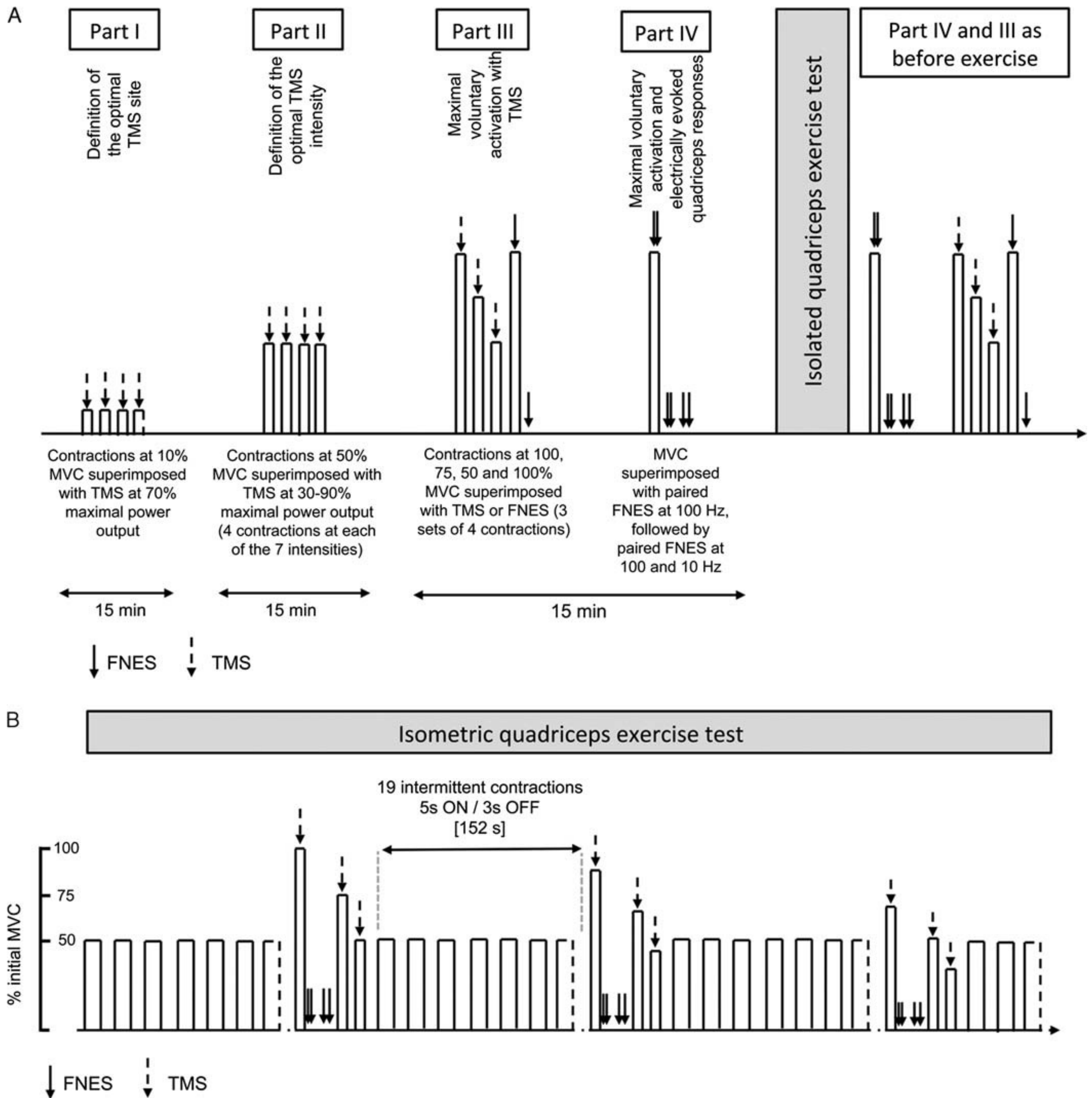

FIGURE 1—Overview of neuromuscular evaluation (A) and isometric knee extension test (B). After standardized warm-up and MVC, neuromuscular evaluation was performed: Part I, determination of the optimal site for TMS; Part II, determination of the optimal TMS intensity; Part III, assessment of maximal VA with TMS; Part IV, assessment of maximal VA and muscle contractility with FNES. The isometric knee extension test consisted in sets of 19 submaximal contractions interspaced by neuromuscular evaluations until TF.

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Neuromuscular evaluations. After EMG electrode placement and determination of FNES intensity (see later discussion), neuromuscular evaluation was performed as summarized in Figure 1A. After a standardized warm-up (i.e., twenty 5-s isometric knee extensor contractions with intensities self-adjusted by the subject to progressively reach MVC during the last three trials), neuromuscular evaluation before the exercise test consisted in the following: I) determination of the optimal TMS site; II) determination of the optimal TMS intensity (recruitment curve); III) sets of VA to assess cortical VA (VA assessed by TMS (VA_{TMS})), motor-evoked potential (MEP; which can infer corticospinal excitability when normalized to M_{max}), and cortical silent period (CSP; i.e., an index of intracortical inhibition); IV) supramaximal FNES paired pulses delivered during and 2 s after MVC to assess peripheral VA (VA assessed by FNES (VA_{FNES})) and knee extensor contractile properties. For every 19 contractions during the exercise test, neuromuscular evaluations consisted of one MVC and two submaximal contractions to determine VA_{TMS}, MEP, CSP (as in Part III), and supramaximal FNES paired pulses 2 s after MVC to determine knee extensor contractile properties (as in Part IV). At TF, neuromuscular evaluation consisted of Parts IV and III as before exercise. Neuromuscular data from one subject were not available due to a technical problem.

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Electrical nerve stimulation. Electrical stimulation was delivered percutaneously to the femoral nerve via a self-adhesive electrode (20-mm-diameter Ag–AgCl; Controle Graphique Medical, Brie-Comte-Robert, France) manually pressed by an experimenter into the femoral triangle to minimize stimulus intensity and discomfort. The anode (a 10 × 5-cm self-adhesive stimulation electrode; Medicompex SA, Ecublens, Switzerland) was located in the gluteal fold. For single and paired stimulations (see later discussion), square wave pulses (1 ms in duration) were produced via a high-voltage (maximal voltage, 400 V) constant-current stimulator (Digitimer DS7; Digitimer, Hertfordshire, UK). FNES intensity (101 ± 21 mA) corresponded to 140% of the optimal intensity (i.e., the stimulus intensity at which the maximal amplitude of twitch force and concomitant quadriceps muscle M-wave were reached). Supramaximal FNES was delivered i) during MVC (paired high-frequency stimuli at 100 Hz; Part IV of neuromuscular evaluation); ii) 2 s after MVC in relaxed muscle as paired high-frequency/low-frequency (100 and 10 Hz) stimuli separated by a 5-s interval (Part IV and neurophysiological evaluations during the exercise test); and iii) during and 2 s after the last MVC of the four contraction sets as single-pulse FNES to obtain M-wave (Part III).

Transcranial magnetic stimulation. A magnetic stimulator (Magstim 200; The Magstim Company, Dyfed, UK) was used to stimulate the motor cortex. Single TMS pulses 1 ms in duration were delivered via a concave double-cone coil (diameter, 110 mm; maximal output, 1.4 T) 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 leg) and to elicit the largest MEP in the RF and VL, with only a small MEP in the BF, during isometric knee extension at 10% of MVC with a stimulus intensity of 70% of maximal stimulator power output (Part I of neuromuscular evaluation; Fig. 1A). 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 the reproducibility of stimulus conditions for each subject throughout the entire session. After 3 min of rest, TMS during brief (3 s) isometric knee extensions at 50% of MVC (Part II) (i.e., the force level inducing the largest MEP) (30) was performed at 30%, 40%, 50%, 60%, 70%, 80%, and 90% 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 (60% ± 13% of stimulator maximal power output) that elicited the largest right RF MEP (i.e., 64% ± 26% RF M-wave) with a small MEP of the right BF (amplitude <10% of maximal RF M-wave) was considered optimal and was employed throughout the protocol, as previously suggested (39). After another 5 min of rest, VA_{TMS} assessment (Part III) consisted of three sets of four brief (3 s) contractions at 100%, 75%, 50% (calculated from the first MVC of each set), and 100% of MVC, with 10 s of rest between contractions and 30 s of rest between series (30). TMS was delivered during the first three contractions, and FNES (single pulse) was delivered during and 2 s after the last contraction. VA_{TMS} during the isometric knee extension test was assessed from one set of three brief (3 s) contractions at 100%, 75%, and 50% of MVC. Strong verbal encouragement was given during MVC, and real-time visual feedback of target force levels was provided to the subjects via custom software (Labview 8; National Instrument) on a computer screen throughout the experiment.

Electromyographic recordings. EMG signals of the right VL, RF, and BF (as a surrogate for antagonist hamstring muscles) were recorded, using bipolar silver chloride surface electrodes 20 mm in diameter (Contrôle Graphique Medical), during voluntary contractions and electrical/magnetic stimuli. The recording electrodes were secured lengthwise to the skin over the muscle belly following SENIAM (Surface Electromyography for the Non-Invasive Assessment of Muscles) recommendations (16), with an interelectrode distance of 25 mm. The positions of EMG electrodes on the skin were marked with indelible ink on the first experimental session to ensure that they were placed at the same location in subsequent visits. The reference electrode was fixed over the patella. 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, bandpass-filtered (5 Hz–1 kHz; input impedance, 200 MΩ; common mode rejection ratio, 85 dB; gain, 1000), recorded at a sampling rate of 2 kHz using BioAmp and PowerLab systems (ADInstruments, Bella Vista, Australia), and stored on a computer for subsequent analysis with LabChart 7 software (ADInstruments).

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NIRS measurements. Changes in oxyhemoglobin (HbO_2), deoxyhemoglobin (HHb), and total hemoglobin (HbTot) concentrations 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, Elst, The Netherlands). Quadriceps muscle hemodynamics was assessed from the right VL using an interoptodes distance of 4 cm. A 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 prefrontal cortex hemodynamics was assessed between Fp1 and F3 according to the international 10–20 electroencephalogram system with 3.5-cm interoptodes 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-wide moving Gaussian smoothing algorithm before analysis.

Cardiorespiratory parameters and RPE. Heart rate, SpO_2 by pulse oximetry at the ear lobe, and $PetCO_2$ from a cannula connected to the face mask were measured continuously (iPM9800; Mindray, Shenzhen, China). Subjects were asked to report their RPE (i.e., how hard they perceived the exercise) at the end of each 19-contraction set and at TF using a 100-mm visual analog scale, with "no effort" on one end (0 mm) and "maximal effort" on the other end (100 mm).

Data analysis. Torque amplitudes of potentiated single-pulse electrical stimulation (T_w) and paired-pulse electrical stimulation at 100 and 10 Hz (Db100 and Db10, respectively) were determined. The presence of low-frequency fatigue (LFF) during and after the isometric knee extension test was evaluated from changes in the Db10-to-Db100 ratio (43). Before and after exercise, the M-wave peak-to-peak amplitude in relaxed muscles (M_{max}) was measured from single-pulse FNES. During exercise, M_{max} was measured from the first stimuli of 10-Hz FNES paired pulses. VA_{FNES} was assessed by twitch interpolation using the superimposed and potentiated doublet amplitudes elicited by 100-Hz FNES paired pulses during and after MVC and calculated from the equation: $VA_{FNES} = [1 - (\text{superimposed 100-Hz FNES paired-pulse amplitude} \times Db100^{-1})] \times 100$.

MEP peak-to-peak amplitudes of quadriceps muscles during TMS superimposed on submaximal and maximal contractions were normalized to M_{max} peak-to-peak amplitude at the same evaluation time point. The duration of CSP was determined visually and defined as the duration from the stimulus to the return of continuous voluntary EMG (15,31). VA_{TMS} was quantified by measuring force responses to TMS. Because motor cortex and spinal cord excitability increase during voluntary contractions, it is necessary to estimate, rather than directly measure, the amplitude of the resting twitch evoked by motor cortex TMS (41). The mean superimposed twitch (SIT) amplitude evoked during contractions at 100%, 75%, and 50% of MVC was calculated, and the y-intercept of the linear regression between mean SIT and voluntary force was used to quantify the estimated resting twitch (ERT). When linear regressions were not linear ($r < 0.9$), ERT was excluded and VA_{TMS} was not calculated for the considered set of contractions (17). ERT was linear for all subjects for at least one set before the exercise test and at TF, permitting VA_{TMS} to be determined in all subjects. During the exercise test, when the linear regression was <0.9 (in 10% of the measurements), VA_{TMS} was extrapolated as the average value between VA_{TMS} values immediately before and VA_{TMS} values immediately after. Cortical VA (%) was calculated using the equation: $VA_{TMS} = [1 - (SIT \times ERT^{-1})] \times 100$. This method has been validated for knee extensors (12,30).

Peak forces measured during stimulations, MEP, CSP, M-waves, and VA before and after the isometric knee extension test were calculated as average values obtained during the three sets of contractions. Mean EMG root mean square (RMS) during the isometric knee extension test was calculated for each 19-contraction set and normalized to M_{max}. HbO_2, HHb, and HbTot concentrations are changes from the initial normoxic baseline value for each experimental session (i.e., 64 ± 1 min before the start of the isometric knee extension test).

Data from the three experimental sessions were compared at four time points: i) before the isometric knee extension test; ii) at 50% of the duration of the shortest exercise test for a given subject (from the three experimental sessions); iii) at 100% of the duration of the shortest exercise test for a given subject (from the three experimental sessions); and iv) at TF. If no neuromuscular evaluation corresponded to exactly 50% or 100% of the duration of the shortest test for a given subject, the nearest neuromuscular evaluation was considered.

Statistical analysis. All statistical procedures were completed on Statistica version 10 (Statsoft, Tulsa, OK, USA). Normality of distribution and homogeneity of variances of the main variables were confirmed using skewness–kurtosis normality test and Levene's test, respectively. Two-way ANOVA (session × time) with repeated measures was performed for each dependent variable. TF was compared among the three experimental sessions using one-way ANOVA with repeated measures. *Post hoc* Tukey's test was applied to determine the difference between two mean values if ANOVA revealed a significant main effect or interaction effect. For all statistical analyses, a two-tailed α level of 0.05 was used as the cutoff for significance. All data are presented as mean ± SD in the text and tables and as mean ± SEM in the figures.

RESULTS

Exercise performance. Exercise duration to TF was significantly shorter in hypoxia with CO_2 clamping (997 ± 460 s) or hypoxia without CO_2 clamping (929 ± 412 s) compared to normoxia (1473 ± 876 s; main effect of experimental condition, $F = 5.5$, $P = 0.010$). No significant differences in performance were found between the two hypoxic conditions.

Cardiorespiratory parameters and sensation. Changes in SpO_2 and $PetCO_2$ during exercise under each

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experimental condition are shown in Figure 2. According to the protocol design, SpO_2 was significantly lower throughout exercise in hypoxia with CO_2 clamping (80% ± 3%) or hypoxia without CO_2 clamping (80% ± 2%) than in normoxia (98% ± 1%; main effect of experimental condition, F = 506.3, P < 0.001), with no difference between hypoxic conditions. $PetCO_2$ was successfully maintained near 40 mm Hg in hypoxia with CO_2 clamping throughout exercise (40 ± 2 mm Hg), whereas lower values were observed at TF in normoxia, and at 100% of the shortest test duration and at TF in hypoxia without CO_2 clamping (main effect of experimental condition, F = 10.3, P < 0.001). Heart rate was significantly higher in hypoxia with CO_2 clamping (109 ± 14 bpm) or hypoxia without CO_2 clamping (109 ± 17 bpm) compared to normoxia (97 ± 16 bpm; main effect of experimental condition, F = 11.1, P < 0.001) throughout exercise, with no significant differences between hypoxic conditions.

Significant main effects of experimental condition (F = 3.8, P = 0.036) and experimental condition–time interaction (F = 3.0, P = 0.010) were found for RPE. RPE was higher in hypoxia with CO_2 clamping than in normoxia at 50% of the shortest test duration (53 ± 25 vs 40 ± 24 mm) and at 100% of the shortest test duration (87 ± 17 vs 75 ± 21 mm). RPE was not significantly different between hypoxic conditions throughout exercise and between all conditions at TF (data not shown).

Prefrontal cortex and *quadriceps femoris* oxygenation. Changes in prefrontal cortex and quadriceps HbO_2, HHb, and HbTot concentrations during exercise under each experimental condition are shown in Figure 3. Prefrontal cortex (main effect of experimental condition, F = 28.0, P < 0.001; experimental condition–time interaction, F = 17.2, P < 0.001) and quadriceps (main effect of experimental condition, F = 21.6, P < 0.001; experimental condition–time interaction, F = 10.7, P < 0.001) HbO_2 concentrations were lower under both hypoxic conditions than under normoxia throughout exercise and were significantly higher in hypoxia with CO_2 clamping than in hypoxia without CO_2 clamping at 100% of the shortest test duration and at TF. Prefrontal cortex (main effect of experimental condition, F = 221.5, P < 0.001; experimental condition–time interaction, F = 56.3, P < 0.001) and quadriceps (main effect of experimental condition, F = 30.8, P < 0.001; experimental condition–time interaction, F = 12.1, P < 0.001) HHb concentrations were higher under both hypoxic conditions compared to normoxia throughout exercise, without a significant difference between hypoxia with CO_2 clamping and hypoxia without CO_2 clamping. Prefrontal cortex HbTot (experimental condition–time interaction, F = 3.7, P = 0.001) concentration was significantly higher in hypoxia with CO_2 clamping than in normoxia at 100% of the shortest test duration compared to hypoxia without CO_2 clamping at TF. Muscle HbTot (experimental condition–time interaction, F = 3.2, P = 0.003) concentration was significantly higher in hypoxia with CO_2 clamping than in hypoxia without CO_2 clamping at 100% of the shortest test duration and at TF.

Neuromuscular fatigue. Changes in maximal and evoked quadriceps force are provided in Table 1. MVC changes were not significantly different among the three experimental conditions (experimental condition–time interaction, F = 2.1, P = 0.064). Db10 at 50% and 100% of the shortest test duration (experimental condition–time interaction, F = 4.7, P < 0.001) and Db100 at 100% of the shortest test duration (experimental condition–time interaction, F = 3.4, P = 0.006) were significantly lower in hypoxia with CO_2 clamping than in normoxia. The Db10-to-Db100 ratio in hypoxia with CO_2 clamping was significantly lower compared to hypoxia without CO_2 clamping at 100% of the shortest test duration and at TF compared to normoxia at 100% of the shortest test duration (experimental condition–time interaction, F = 2.3, P = 0.047; Fig. 4A). T_w in hypoxia with CO_2 clamping was also significantly lower compared to hypoxia without CO_2 clamping at TF (experimental condition–time interaction, F = 2.2, P = 0.041). M_{max} increased similarly during exercise under all experimental conditions (all P > 0.05; Table 2).

VA_{TMS} in hypoxia without CO_2 clamping decreased to a larger extent at TF compared to hypoxia with CO_2 clamping

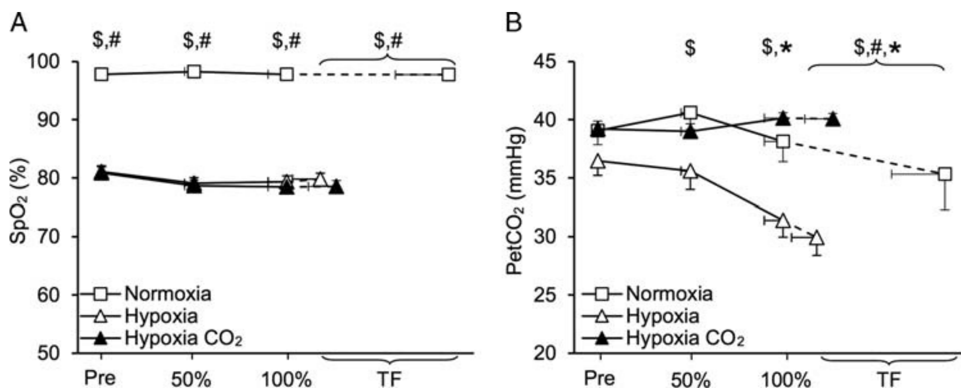

FIGURE 2—SpO_2 (A) and $PetCO_2$ (B) during the isometric knee extension test in normoxia (Normoxia), hypoxia with CO_2 clamping (Hypoxia CO_2), or hypoxia without CO_2 clamping (Hypoxia). Data points are presented as mean ± SEM (*n* = 15). Significant differences between $Normoxia and Hypoxia, #Normoxia and Hypoxia CO_2, and *Hypoxia and Hypoxia CO_2. Pre, before exercise; 50%, 50% of the duration of the shortest test; 100%, 100% of the duration of the shortest test.

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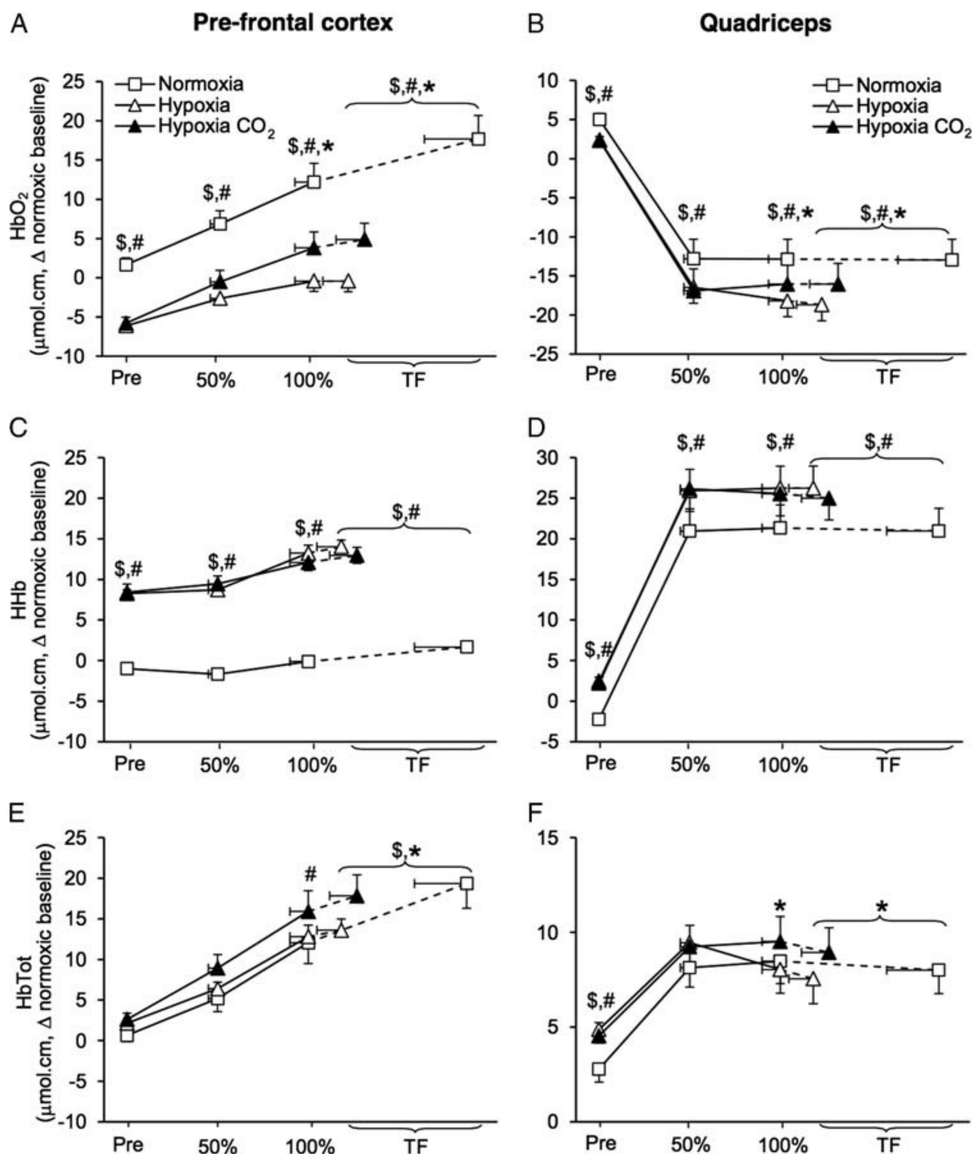

FIGURE 3—Prefrontal cortex and quadriceps HbO_2 (A and B), HHb (C and D), and HbTot (E and F) during the isometric knee extension test in normoxia (Normoxia), hypoxia with CO_2 clamping (Hypoxia CO_2), or hypoxia without CO_2 clamping (Hypoxia). Data points are presented as mean ± SEM (n = 15). Significant differences between $Normoxia and Hypoxia, #Normoxia and Hypoxia CO_2, and *Hypoxia and Hypoxia CO_2. Pre, before exercise; 50%, 50% of the duration of the shortest test; 100%, 100% of the duration of the shortest test.

and normoxia (experimental condition–time interaction, $F = 3.5$, $P = 0.004$; Fig. 4B). VA_{FNES} decreased similarly at TF under all experimental conditions (experimental condition–time interaction, $F = 0.7$, $P = 0.506$; Table 1). No significant difference between experimental conditions was observed for $MEP{\cdot}M_{max}^{-1}$ and CSP throughout exercise at any force level (50% of MVC, Table 2; 75% and 100% of MVC, results not shown; all $P > 0.05$). Mean RF $RMS{\cdot}M_{max}^{-1}$ during exercise was similar between experimental conditions (experimental condition–time interaction, $F = 0.79$, $P = 0.463$; results not shown). However, VL $RMS{\cdot}M_{max}^{-1}$ was significantly smaller during the set corresponding to 100% of the shortest test duration in normoxia (0.027 ± 0.008) than in hypoxia with CO_2 clamping (0.039 ± 0.009; experimental condition–time interaction, $F = 5.1$, $P = 0.002$). VL $RMS{\cdot}M_{max}^{-1}$ was also significantly smaller during the last set of contractions before TF in hypoxia without CO_2 clamping (0.037 ± 0.010) than in normoxia (0.042 ± 0.011; $P < 0.05$) and hypoxia with CO_2 clamping (0.042 ± 0.012).

DISCUSSION

This is the first study to evaluate CO_2 clamping at iso-SpO_2 (80%) on neuromuscular fatigue and the contributing mechanisms during isometric knee extensions performed in hypoxia. The main results are that CO_2 clamping in hypoxia i) enhances cerebral and muscle oxygenation, ii) reduces central fatigue but enhances peripheral muscle fatigue, and

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TABLE 1. Quadriceps peak force during MVC, T_w, paired-pulse electrical stimulation (at 10 Hz (Db10) and 100 Hz (Db100)), and quadriceps VA_{FNES} during the isometric knee extension test in normoxia (Normoxia), hypoxia with CO_2 clamping (Hypoxia CO_2), or hypoxia without CO_2 clamping (Hypoxia) (n = 14).

		Pre	50%	100%	TF
MVC (N)	Normoxia	475.0 ± 7.6	361.7 ± 5.2	320.1 ± 8.2	281.5 ± 5.9
	Hypoxia	470.0 ± 7.8	335.1 ± 5.1	305.1 ± 5.3	294.0 ± 5.0
	Hypoxia CO_2	469.9 ± 7.8	342.2 ± 6.8	285.7 ± 6.0	288.5 ± 6.6
Db10 (N)	Normoxia	190.5 ± 3.9	126.6 ± 4.4	100.0 ± 4.5	84.4 ± 4.2
	Hypoxia	208.5 ± 5.3	113.9 ± 4.0	89.7 ± 4.2	89.1 ± 4.0
	Hypoxia CO_2	203.4 ± 5.0	103.9 ± 2.5[a]	74.9 ± 2.5[a]	74.6 ± 2.9
Db100 (N)	Normoxia	223.3 ± 4.4	177.5 ± 4.2	154.1 ± 4.7	139.9 ± 4.8
	Hypoxia	232.7 ± 4.1	165.2 ± 4.6	138.4 ± 5.1	140.1 ± 5.2
	Hypoxia CO_2	232.2 ± 4.0	160.3 ± 3.4	132.3 ± 2.7[a]	133.5 ± 3.2
T_w (N)	Normoxia	148.7 ± 24.7	—	—	76.7 ± 22.5
	Hypoxia	145.4 ± 27.1	—	—	83.4 ± 28.8
	Hypoxia CO_2	147.5 ± 27.2	—	—	67.7 ± 22.5[b]
VA_{FNES} (%)	Normoxia	89.3 ± 7.4	—	—	83.2 ± 13.1
	Hypoxia	90.3 ± 6.5	—	—	82.7 ± 15.2
	Hypoxia CO_2	90.3 ± 5.1	—	—	80.5 ± 13.5

Data are presented as mean ± SD.
ANOVA revealed a significant time effect for all variables (P < 0.05).
[a]Significantly different from Normoxia.
[b]Significantly different from Hypoxia.
Pre, before exercise; 50%, 50% of the duration of the shortest test; 100%, 100% of the duration of the shortest test.

iii) has no significant effects on performance compared to hypoxia without CO_2 clamping. These results confirm that hypocapnia during hypoxic exercise has a significant effect on tissue oxygenation and demonstrate that CO_2 clamping influences central and peripheral determinants of neuromuscular fatigue. Taken together, our findings provide valid explanations as to why CO_2 clamping does not change exercise performance in hypoxia.

Methodological considerations. The protocol design of the present study aimed to avoid several limitations of previous studies that investigated the effects of CO_2 clamping during exercise in hypoxia. First, we aimed to avoid important potential consequences of enhanced ventilatory response elicited by CO_2 clamping (i.e., an increase in arterial blood oxygenation) (7,8,37) and potential deleterious effects of adverse respiratory sensations and large respiratory muscle work on performance (6). We successfully maintained SpO_2 at 80% under both hypoxic conditions in all subjects; therefore, we were able to control the effects of CO_2-induced ventilatory stimulation and the large SpO_2 intersubject heterogeneity observed with fixed FiO_2. This level of hypoxemia and the reduction in performance were similar to previous results with the same exercise modality and FiO_2 (0.10) (13). In the present study, $PetCO_2$ was used as a surrogate for arterial CO_2 in order to clamp CO_2 at 40 mm Hg. Although end-tidal–arterial PCO_2 gradient is known to be modified with exercise (18), studies with a similar setup as the present one confirmed the ability of CO_2 clamping (based on $PetCO_2$) to keep arterial CO_2 constant (7,32). With one-leg isometric knee extensions, cardiorespiratory responses to exercise were modest, as shown by heart rate levels and previous measurements of minute ventilation at similar exercise settings (e.g., (13)). Hence, we are confident that the consequence of CO_2-induced increase in respiratory muscle work at relatively low levels of ventilation was unlikely to significantly affect exercise performance.

Because the potential interference of cutaneous circulation with cerebral and muscle NIRS measurements is an important concern (34), one should wonder whether differences in NIRS signals between hypoxia with CO_2 clamping and

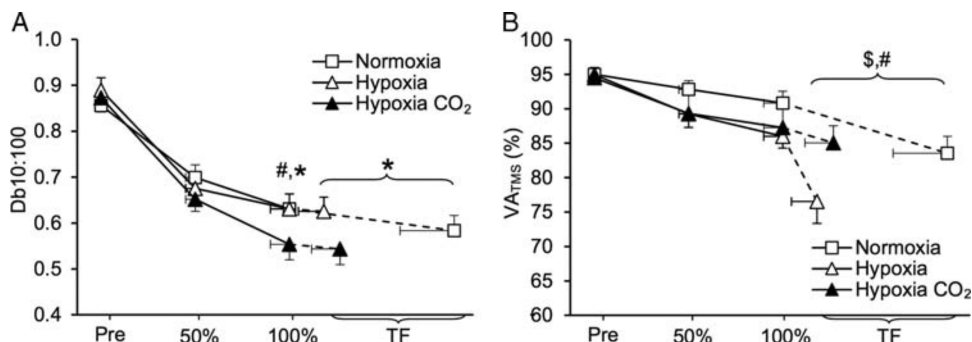

FIGURE 4—Ratio of paired-pulse peak force at 10 and 100 Hz (Db10:100) in knee extensor muscles (A) and maximal VA_{TMS} (B) during the isometric knee extension test in normoxia (Normoxia), hypoxia with CO_2 clamping (Hypoxia CO_2), or hypoxia without CO_2 clamping (Hypoxia). Data points are presented as mean ± SEM (n = 14). Significant differences between $Normoxia and Hypoxia, #Normoxia and Hypoxia CO_2, and *Hypoxia and Hypoxia CO_2. Pre, before exercise; 50%, 50% of the duration of the shortest test; 100%, 100% of the duration of the shortest test.

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TABLE 2. VL and RF M-wave (M_{max}), MEP (during voluntary contractions at 50% of MVC) amplitudes, and CSP (during voluntary contractions at 50% of MVC) during the isometric knee extension test in normoxia (Normoxia), hypoxia with CO_2 clamping (Hypoxia CO_2), or hypoxia without CO_2 clamping (Hypoxia) (n = 14).

		Pre	50%	100%	TF
M_{max} (mV)					
VL	Normoxia	13.7 ± 3.9	14.1 ± 4.1	14.3 ± 4.2	14.7 ± 4.0
	Hypoxia	14.4 ± 3.7	14.8 ± 3.3	15.3 ± 3.3	16.0 ± 3.7
	Hypoxia CO_2	13.3 ± 3.4	13.1 ± 3.7	13.8 ± 3.8	15.0 ± 3.4
RF	Normoxia	5.2 ± 1.0	5.1 ± 1.0	5.4 ± 1.0	5.2 ± 0.9
	Hypoxia	5.5 ± 1.3	5.8 ± 1.5	6.0 ± 1.5	6.1 ± 1.6
	Hypoxia CO_2	5.4 ± 1.4	5.4 ± 1.5	5.7 ± 1.7	5.8 ± 1.8
MEP·M_{max}^{-1}					
VL	Normoxia	39.1 ± 12.4	44.6 ± 14.8	45.0 ± 10.2	38.7 ± 10.7
	Hypoxia	43.8 ± 13.2	47.5 ± 15.0	39.0 ± 13.2	41.2 ± 13.1
	Hypoxia CO_2	41.1 ± 10.0	51.2 ± 15.2	46.6 ± 12.7	42.3 ± 12.3
RF	Normoxia	66.2 ± 22.7	69.8 ± 26.7	64.2 ± 30.5	61.9 ± 32.0
	Hypoxia	64.0 ± 20.9	71.9 ± 24.1	67.0 ± 28.6	66.1 ± 27.3
	Hypoxia CO_2	61.8 ± 18.9	72.7 ± 24.2	67.1 ± 26.6	65.6 ± 24.3
CSP (ms)					
VL	Normoxia	238 ± 75	248 ± 64	253 ± 73	235 ± 71
	Hypoxia	223 ± 66	206 ± 94	216 ± 89	233 ± 88
	Hypoxia CO_2	237 ± 68	213 ± 89	205 ± 100	234 ± 75
RF	Normoxia	243 ± 75	240 ± 82	254 ± 66	234 ± 52
	Hypoxia	229 ± 73	218 ± 99	208 ± 110	231 ± 77
	Hypoxia CO_2	227 ± 73	219 ± 90	203 ± 98	238 ± 73

Data are presented as mean ± SD.
ANOVA revealed a significant time effect for all variables (P < 0.05), except CSP.
Pre, before exercise; 50%, 50% of the duration of the shortest test; 100%, 100% of the duration of the shortest test.

hypoxia without CO_2 clamping may arise from the effects of CO_2 on cutaneous circulation. This seems unlikely, however, because Simmons et al. (33) previously demonstrated that changes in systemic CO_2 do not affect cutaneous circulation under conditions similar to the present study (i.e., SpO_2 ~ 80%). One should also acknowledge that cerebral NIRS was used over the prefrontal cortex only and therefore did not take into account potential regional differences in hypoxic and CO_2 cerebrovascular responses. Some data suggest that the prefrontal cortex and motor cortex may exhibit different changes in oxygenation during normoxic and hypoxic exercises (19,26,36). Lastly, no measurement of cerebral blood flow or velocity was available in the present study. Because of the important effects of hypoxia on cerebral blood flow regulation (24), additional studies with transcranial Doppler, including assessment of potential regional differences (e.g., between the anterior cerebral circulation and the posterior cerebral circulation), are needed to confirm the data that we obtained with NIRS.

Effects of CO_2 clamping on tissue oxygenation. Asexpected and as previously reported (7,28,35), cerebral and muscle oxygenation were significantly reduced in hypoxia. CO_2 clamping significantly increased HbO_2 and HbTot in the prefrontal cortex and quadriceps toward the end of hypoxic exercise. These differences were observed at 100% of the shortest test duration and at TF (i.e., at the time points when significant differences in $PetCO_2$ were observed between hypoxia with CO_2 clamping and hypoxia without CO_2 clamping), supporting the link between arterial CO_2 and tissue oxygenation differences between the two hypoxic conditions. Although vasoreactivity to CO_2 is known to be greater in cerebral vasculature than in muscle vasculature (1), the present results indicate that an average increase in $PetCO_2$ of 10 mm Hg (i.e., the average difference between hypoxia with CO_2 clamping and hypoxia without CO_2 clamping toward the end of exercise) significantly enhances prefrontal cortex and quadriceps HbO_2 and HbTot concentrations. This muscular effect of CO_2 clamping in hypoxia is in contrast to the unchanged muscle oxygenation with CO_2 clamping assessed by NIRS during a maximal incremental cycling in hypoxia reported by Subudhi et al. (37), whereas Fan et al. (7) recently reported a tendency for increased muscle oxygenation with CO_2 clamping during a 15-km cycling time trial in hypoxia. Hypercapnia increased femoral blood flow at rest, although to a smaller extent than did cerebral blood flow (1). Differences in experimental conditions (rest vs exercise, exercise modality and intensity) might explain these contrasting results regarding the effects of CO_2 clamping on muscle oxygenation.

Oxygen delivery to the brain (3) and muscles (5) is thought to be the main determinant of exercise performance in hypoxia. Although tissue oxygen delivery could not be calculated in the present study because blood flow was not measured, one could suggest that increasing prefrontal cortex and quadriceps oxygenation, as measured by NIRS, during hypoxic exercise with CO_2 clamping would improve exercise performance compared to hypoxic exercise without CO_2 clamping. Despite the effects of CO_2 clamping on tissue oxygenation, time to TF was similar under the two hypoxic conditions. This suggests that either differences in tissue oxygenation with CO_2 clamping were not large enough to significantly affect exercise performance or endurance performance during intermittent isometric quadriceps contractions in hypoxia was not limited by levels of cerebral and muscle oxygenation.

Effects of CO_2 clamping on central drive. Over the past decade, numerous observations regarding neuromuscular responses to hypoxic exercise led to the theory that the effects of hypoxia on the central nervous system are responsible, at least in part, for altered central motor command and, consequently, reduced exercise performance (14,44).

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This theory has been supported by the largest amount of central fatigue measured by TMS following exhaustive whole-body exercise (11) and knee extension exercise (13) in severe hypoxia (SpO_2 ~ 80%) compared to normoxia. In the present study, we confirmed these data by showing that VA_{TMS} at TF was reduced to a greater extent in hypoxia without CO_2 clamping compared to normoxia. This difference in VA_{TMS} occurred together with unchanged indices of corticospinal excitability ($MEP \cdot M_{max}^{-1}$) and intracortical inhibition (CSP). Previous results from our group suggest that a more prolonged hypoxic exposure (several hours) is needed to alter motor cortex excitability (27).

Because the greater alteration in maximal central drive after exercise in hypoxia may be due to a significant reduction in cerebral oxygenation (11,13), we hypothesized that improving cerebral oxygenation during hypoxic exercise by clamping CO_2 would reduce the amount of central fatigue at TF compared to hypoxia without CO_2 clamping. The present results confirm this hypothesis by showing a significantly smaller reduction in VA_{TMS} at TF in hypoxia with CO_2 clamping compared to hypoxia without CO_2 clamping (Fig. 4B). VA_{FNES} did not differ, however, at TF between experimental conditions, suggesting that methodological considerations (e.g., linearity of torque–SIT relationship) may make this measurement of central fatigue less sensitive than VA_{TMS} (31,40,41). A smaller VL $RMS \cdot M_{max}^{-1}$ during the last set of contractions before TF in hypoxia without CO_2 clamping, compared to hypoxia with CO_2 clamping and normoxia, further suggests that CO_2 clamping improved central drive in hypoxia. This happens despite unchanged corticospinal excitability and intracortical inhibition, confirming previous reports of unchanged corticospinal excitability during hyperventilation and hypercapnia at rest (20).

VA_{TMS} reduction and $RMS \cdot M_{max}^{-1}$ at TF in hypoxia with CO_2 clamping were similar to those in normoxia despite cerebral oxygenation being significantly lower due to the hypoxic condition. Previous results suggested that central fatigue is accentuated following exercise performed in severe hypoxia only (3,13,23). For instance, severe hypoxia (FiO_2 = 0.10)—but not moderate hypoxia (FiO_2 = 0.13)—accentuates the reduction in VA_{TMS} following exhaustive intermittent isometric quadriceps contractions (13). Hence, despite CO_2 clamping not normalizing cerebral oxygenation to normoxic level, it increased cerebral oxygenation sufficiently to avoid the alteration in central drive induced by the severe hypoxic condition in the present study (i.e., in hypoxia without CO_2 clamping) and in previous studies (11,13). The significant effect of CO_2 clamping on central fatigue following hypoxic exercise demonstrates that hypocapnia during hypoxic exercise has significant consequences at the cerebral level, leading to reduced central drive to muscles.

Despite the smaller extent of central fatigue with CO_2 clamping in hypoxia, time to TF was similar to that in hypoxia without CO_2 clamping, whereas it was significantly shorter than that in normoxia. This suggests that mechanisms other than central fatigue were responsible for TF in hypoxia with CO_2 clamping (e.g., peripheral mechanisms).

Effects of CO_2 clamping on peripheral fatigue. The assessment of quadriceps muscle fatigue with FNES showed that peripheral fatigue was accentuated in hypoxia with CO_2 clamping compared to hypoxia without CO_2 clamping and normoxia (Table 1, Fig. 4). Changes in the Db10-to-Db100 ratio indicates that LFF was greater at 100% of the shortest test duration and at TF in hypoxia with CO_2 clamping compared to hypoxia without CO_2 clamping. A larger LFF was further supported by a lower T_w amplitude at TF in hypoxia with CO_2 clamping compared to hypoxia without CO_2 clamping. VL $RMS \cdot M_{max}^{-1}$ at "isotime" (during the set of contractions corresponding to 100% of the shortest test duration) was greater in hypoxia with CO_2 clamping compared to normoxia. This suggests that greater central drive was required to sustain the target force level in hypoxia with CO_2 clamping, consistent with an accelerated rate of peripheral fatigue development under this later condition. Subjects reported greater RPE during exercise at isotime in hypoxia with CO_2 clamping compared to normoxia, which might be linked, at least in part, to the larger amount of peripheral muscle fatigue under the former condition.

Respiratory acidosis induced by CO_2 inhalation has been shown to reduce limb muscle contractility and to increase exercise-induced LFF (21,45). Respiratory acidosis may reduce intracellular pH (10), which would subsequently alter muscle contractility (21,45). The effects of CO_2 inhalation on arterial and intracellular pH (as observed in the present study) are, however, unclear, with studies reporting inconsistent changes in arterial pH with CO_2 clamping (7,37,38) and with other studies suggesting that reduced arterial pH may not necessarily translate into reduced muscular pH (e.g., (29)). Furthermore, the effects of reduced intracellular pH on muscle contractility and fatigue have been debated (4,47), suggesting that the link between reduced intramuscular pH and muscle fatigue may not be straightforward. The present study suggests that the lack of exercise performance improvement with CO_2 clamping in hypoxia despite increased cerebral oxygenation (7,8,32,37) was due to the deleterious effect of CO_2 clamping on muscle contractility, overruling the positive effect of CO_2 clamping on cerebral and muscle oxygenation and on central fatigue. Clamping both arterial CO_2 and pH with bicarbonate infusion, for instance (25,46), could allow for the dissociation of the central and peripheral effects of hypocapnia during hypoxic exercise in future studies.

In conclusion, the present results demonstrate that CO_2 clamping during isometric knee extensions in hypoxia modifies the contributions of central and peripheral mechanisms to fatigue in men. CO_2 clamping enhances cerebral oxygenation and reduces central fatigue, whereas it increases LFF despite some improvement in muscle oxygenation. These effects lead to unchanged exercise performance compared to hypoxia without CO_2 clamping. Hence, the present study emphasizes the role of cerebral oxygenation in central drive and its regulation by arterial CO_2 during hypoxic exercise. It

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also suggests that hyperventilation-induced hypocapnia during hypoxic exercise protects muscle function by reducing the amount of peripheral muscle fatigue. Whether similar CO_2 clamping has similar effects on central and peripheral fatigue during isolated muscle exercise and whole-body exercise (e.g., cycling) remains to be assessed.

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