Peripheral fatigue is not critically regulated during maximal, intermittent, dynamic leg extensions

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Christian RJ, Bishop DJ, Billaut F, Girard O. Peripheral fatigue is not critically regulated during maximal, intermittent, dynamic leg extensions. J Appl Physiol 117: 1063–1073, 2014. First published September 11, 2014; doi:10.1152/japplphysiol.00988.2013.—Central motor drive to active muscles is believed to be reduced during numerous exercise tasks to prevent excessive peripheral fatigue development. The purpose of the present study was to use hypoxia to exacerbate physiological perturbations during a novel, intermittent exercise task and to explore the time-course and interplay between central and peripheral neuromuscular adjustments. On separate days, 14 healthy men performed four sets of 6 × 5 maximal-intensity, isokinetic leg extensions (1 repetition lasting ~7 s) at 300°/s (15 and 100 s of passive rest between repetitions and sets, respectively) under normoxia (NM, fraction of inspired O2 0.21), moderate (MH, 0.14), and severe normobaric hypoxia (SH, 0.10). Neuromuscular assessments of the knee extensors were conducted before and immediately after each set. There was an interaction between time and condition on the mean peak torque produced during each set (P < 0.05). RMS/M-wave activity of the rectus femoris decreased across the four sets of exercise, but there was no difference between conditions (8.3 ± 5.1% all conditions compounded, P > 0.05). Potentiated twitch torque decreased post set 1 in all conditions (all P < 0.05) with greater reductions following each set in SH compared with NM but not MH (end-exercise reductions 41.3 ± 3.0% vs. 28.0 ± 3.2%, P < 0.05 and 32.1 ± 3.3%, P > 0.05). In conclusion, severe hypoxia exacerbates both peripheral fatigue development and performance decrements during maximal, intermittent, dynamic leg extensions. In contrast to observations with other exercise modes, during exercise involving a single muscle group the attenuation of central motor drive does not appear to independently regulate the development of peripheral muscle fatigue.

hypoxia; neuromuscular fatigue; time-course; isolated contractions; maximal intermittent exercise

NEUROMUSCULAR FATIGUE can be defined as any reversible, exercise-induced, reduction in maximal force generating capacity (38). The contribution of central and peripheral mechanisms to the development of fatigue depends not only on the intensity, mode, and duration of exercise, but also environmental factors such as altitude (16, 33, 57). Reductions in oxygen availability, or hypoxic conditions, have been well documented to impair exercise capacity during both exhaustive, whole-body continuous (6) or intermittent (15, 24, 72) exercise and during isolated leg extensions (42). As such, hypoxia is often used as a perturbation to exacerbate the rate of fatigue development and to explore the interplay between the central and peripheral mechanisms underpinning neuromuscular fatigue.

Traditionally, the decrease in exercise capacity during whole-body exercise under hypoxic conditions has been largely attributed to the effects of reduced arterial oxygen saturation, and subsequent lower oxygen delivery, on muscle contractility (1, 45). Although mounting evidence suggests that central motor output may also be lowered when exercising under hypoxic conditions (4, 77), it remains to be elucidated whether these exercise-induced reductions occur in parallel with a conscious attenuation of effort or are mediated via subconscious feedback pathways (51). In fact, a reduction in neural drive to the active musculature observed under hypoxic conditions may occur as a consequence of increased afferent feedback from fatigued locomotor muscles inhibiting the motor pathway from the motor cortex to the contracting muscles and/or impairing voluntary descending drive upstream from the motor cortex (12), presumably to restrict the development of excessive peripheral fatigue (4, 12, 13). However, the relative importance of afferent feedback has also been suggested to decrease under acute conditions of severe hypoxia, when reductions in arterial oxygen saturation (70–75% (7, 11, 55)) and/or cerebral oxygenation (8, 13) may pose a direct threat to the central nervous system (CNS) and markedly attenuate both the level of neural drive to active muscles and subsequent peripheral fatigue development (11).

To date, the majority of research investigating the interplay between central and peripheral mechanisms of fatigue have employed exhaustive, whole-body continuous exercise (6, 10) or repeated, brief, submaximal, isometric contractions (42, 47, 54). However, during such submaximal, self-paced exercise models, adjustments in neural drive are likely to be influenced by a conscious pacing strategy (32, 73). Accordingly, psychological factors such as a participant’s level of motivation, previous experience of the task, knowledge of the task duration remaining, and the conscious perception of the sensation of fatigue are likely to be influential (71, 74, 79). Furthermore, anticipatory adjustments in neural drive (63, 81) have been observed even during supramaximal exercise [i.e., exercise intensities above that required to elicit maximal oxygen uptake (56)] when the duration of the task is longer than 30 s (14, 85). However, during maximal cycling sprints of less than 15 s duration, an all-out pacing strategy is likely to be adopted in the absence of any anticipatory preexercise adjustments in neural drive (85). Therefore, short duration, maximal efforts, which are well known to induce substantial levels of peripheral fatigue (20), may provide a more appropriate model for exploring the central regulation of peripheral fatigue development.
With reductions in convective oxygen transport shifting any given absolute workload to a higher relative intensity, identifying the effects of hypoxia on fatigue development during whole-body exercise is further complicated (4, 42, 84). As such, isolated contractions have recently been used as an alternative exercise model to overcome this limitation and allow the investigation of the interplay between central and peripheral mechanisms of fatigue to be explored independently of hypoxia-induced shifts in the relative intensity of exercise (28, 84). Furthermore, isolated contractions also avoid any confounding effects that inspiratory muscle fatigue might have on reducing limb blood flow and accelerating fatigue development via the sympathetically mediated metaboreflex (9, 30).

An additional limitation of whole-body, continuous exercise models is that neuromuscular assessments are often restricted to only pre- and postexercise, and thus provide very limited information about the time-course of neuromuscular adjustments. Alternatively, maximal intensity intermittent exercise (MIIE), characterized by brief, maximal exercise bouts interspersed with longer, but incomplete, recovery periods (e.g., repeated sprinting) allows the kinetics of central and peripheral fatigue development to be assessed during the exercise task itself. Despite this advantage, hypoxia has not been used to explore the interplay between central and peripheral fatigue development during repeated, short-duration, maximal dynamic exercise of an isolated muscle group. Furthermore, with the recovery of neuromuscular function shown to occur rapidly, within the first minute of terminating exercise (35), using an intermittent, isolated task (completed on the same ergometer as the neuromuscular assessment) presents the opportunity to almost immediately (within the first 15 s) evaluate the magnitude and etiology of muscle fatigue developed during repeated, short duration, maximal exercise the moment exercise ceases.

To explore the kinetics of central and peripheral fatigue development during repeated short-duration maximal efforts in a closed-loop design [exercise of set duration (75)], we assessed neuromuscular function at regular intervals during the recovery periods of maximal, intermittent, dynamic contractions conducted under various conditions of inspired oxygen fraction. Furthermore, to explore the presence of any switch in the determinants of central neural regulation under severe hypoxic conditions, we utilized three different levels of hypoxia severity, which has never been used during repeated, maximal-intensity, short-duration exercise. With previous studies reporting similar end-exercise levels of peripheral fatigue across a range of interventions (e.g., mild-moderate hypoxia, precooling, preexisting locomotor muscle fatigue) (5, 6, 9, 11, 31, 36, 64), we hypothesized that hypoxia severity would exacerbate the initial rate of peripheral fatigue development, yet the end-exercise level of peripheral fatigue would not be different across normoxic and moderate hypoxic conditions due to an exacerbated decline in central motor output mediated via increased afferent feedback under moderate hypoxic conditions. Furthermore, we hypothesized that severe arterial oxygen desaturation and associated prefrontal cortex oxygenation under severe hypoxic conditions would be accompanied by earlier and larger reductions in central motor drive ultimately leading to a markedly attenuated development of end-exercise peripheral fatigue.

METHODS

Participants. Fourteen moderately trained, team-sport athletes accustomed to high-intensity, intermittent exercise, volunteered for this study (mean ± SD age 29.4 ± 5.0 yr, stature 1.80 ± 0.38 m, body mass 82.6 ± 7.1 kg). All participants gave written, informed consent before the commencement of the study after all the experimental procedures, associated risks, and potential benefits of participation had been explained. The study was approved by the Victoria University Human Research Ethics Committee. All procedures conformed to the Declaration of Helsinki. Participants were asked to avoid vigorous exercise for 24 h, caffeine for 12 h, and food for 2 h before every trial.

Experimental design. All participants performed one familiarization session and three experimental trials in a randomized, single-blind design. Participants reported to the laboratory 1 wk prior to the first experimental session and were familiarized with all experimental procedures. Particular attention was paid to familiarization of the maximal voluntary contraction (MVC) of the knee extensors, with participants repeating the procedures until they were able to maintain a stable plateau in torque for 3–4 s and produce consistent values (CV less than 5%). Participants were also fully familiarized with peripheral motor nerve stimulation and the fatiguing exercise by completing two full sets of the exercise protocol (see Exercise protocol).

During the experimental sessions, participants were exposed to three conditions of simulated altitude/inspired O2 fraction (FiO2) [normoxia (NM) 0 m/0.21, moderate hypoxia (MH) 3,000 m/0.14, and severe hypoxia (SH) 5,400 m/0.10] with all tests completed in a normobaric hypoxic chamber (Colorado Mountain Room System; Colorado Altitude Training, Boulder, CO). The experimental trials were separated by at least 5 days and performed at the same time of day to avoid possible effects of circadian rhythm (4, 84, 86). Upon entrance, participants rested in a seated position for 10 min (wash-in period) while all equipment was attached. The preexercise assessment of neuromuscular function was then made, followed by a standardized warm up and the exercise protocol. Neuromuscular assessments were completed before and immediately after each set of exercise (see Exercise protocol).

Exercise protocol. The exercise protocol consisted of four sets of 6 × 5 continuous maximal isokinetic leg extensions of the dominant leg. Each leg extension started from 90° of knee flexion and was conducted though 70° of motion at 300°/s (lasting ~235 ms). The dynamometer arm then automatically returned the leg back through 90° of passive knee flexion at ~70°/s (lasting ~1 s) to begin the next leg extension. Each group of five continuous leg extension/passive flexion actions, classified as one repetition, lasted ~7 s and was separated by 15 s of passive rest. Sets were separated by 100 s. Prior to the exercise, subjects completed a dynamic warm-up consisting of one set of 6 × 5 continuous isokinetic leg extensions. During the warm-up, the subjective effort of each leg extension was progressively increased by 10%, from 50% to 100% of perceived maximal effort. Following the warm-up procedure participants rested passively for 2 min before beginning the exercise protocol.

Responses to exercise. Heart rate (HR), monitored via a wireless Polar monitoring system (Polar Electro Oy, Kempele, Finland) and arterial oxygen saturation (SpO2), estimated noninvasively via pulse oximetry using a finger probe (Palmstat 2500; NONINMedical, Plymouth, MI), were recorded immediately prior to entering the chamber and following the 10 min wash-in period. Additionally, HR, SpO2, and a measure of the rating of perceived exertion (RPE), based on the 6–20 Borg scale, with participants rating their overall perception of how hard the exercise felt, were obtained exactly 10 s after each repetition.

Torque and electromyographic recordings. All neuromuscular assessments and the exercise protocol were conducted on an isokinetic dynamometer (Biodex; Isokinetic Dynamometer, Shirley, NY). Participants were seated (90° hip extension) and securely strapped into the chair. Movements of the upper body were limited by two cross-
over shoulder harnesses and a belt across the abdomen. The axis of the dynamometer was aligned with the knee flexion-extension axis, and the lever arm was attached to the shank around the ankle with a strap. Electromyographic (EMG) signals of the rectus femoris muscle were recorded via bipolar Ag/AgCl electrodes (Ambu Blue sensor T; Ambu A/S, Denmark) with a diameter of 9 mm and an interelectrode distance of 3 cm. Before electrode placement, the skin was lightly abraded and washed to remove surface layers of dead skin, hair, and oil. The ground electrode was attached to the right wrist. The position of the EMG electrodes was marked with indelible ink (and photographs of the locations were taken) to ensure that they were placed in the same location during subsequent trials. To ensure low levels of movement artifact, electrode cables were fastened to subjects’ bodies with medical adhesive tape and wrapped in net. The myoelectric signal was amplified (gain = 1,000×) and filtered (bandwidth frequency = 12 to 500 Hz) to minimize extraneous noise and possible movement artifacts in the low-frequency region and to eliminate aliasing and other artifacts in the high-frequency region. Torque and EMG signals were recorded (sampling frequency = 2,000 Hz) using MP35 hardware (Biopac Systems, Santa Barbara, CA) and dedicated software (Biopac Pro Version 3.6.7; Biopac Systems).

Femoral nerve stimulation. A high-voltage, constant current, stimulator (Digitimer DS7AH; Digitimer, Hertfordshire, UK) was used to deliver a rectangular pulse of 200 μs with maximal voltage of 400 V to the femoral nerve. A monopolar cathode ball electrode (0.5 cm diameter) was manually pressed into the femoral triangle (i.e., 3–5 cm below the inguinal ligament) by the experimenter. The anode, a self-adhesive pad (5 × 10 cm, Medicomplex; Eculbens, Switzerland) was located in the gluteal fold opposite the cathode. During the familiarization session a passive isometric recruitment curve was drawn. Briefly, the stimulation intensity was progressively increased by 10 mA increments until plateaus occurred in twitch amplitude and compound muscle action potential (M-wave). Supramaximal stimulation was ensured by increasing the final intensity by 30% (mean current 95 ± 6 mA).

Neuromuscular function. During the neuromuscular assessments the knee angle was fixed at 90° of flexion (0° corresponding to full knee extension). The neuromuscular assessment consisted of a 4-s MVC of the knee extensors with a superimposed 20 Hz doublet (Db) applied to the peripheral motor nerve when torque had reached a visible plateau. This was followed after 3 s by 1) one 20 Hz Db, 2) one 80 Hz Db, and 3) three single twitches in a relaxed state (all separated by 3 s). This neuromuscular testing was conducted three times preexercise and once exactly 15 s after each of the four exercise sets (post set 1, post set 2, post set 3, and post set 4). Prior to the preexercise neuromuscular assessment subjects were warmed up by completing 5 × 4-s voluntary isometric contractions with progressively increasing subjective effort (starting at 50% of subjective maximal effort with increments of 10%, 20 s of passive rest separated each contraction) followed by 2 × 4-s MVC (separated by 1 min of passive rest).

Data analysis. All torque and EMG data were analyzed using a dedicated analysis system (Spoke2 v3.21; Cambridge Electronic, Cambridge Design, Cambridge, UK). The mean peak torque for each repetition (five knee extensions) was calculated as the average peak torque produced during each isokinetic contraction, giving one value for each repetition to be considered for further analysis. The mean peak torque for each set (six repetitions, e.g., set 1) was calculated as the average mean peak torque during each repetition, with the torque percentage decrement score for the entire exercise protocol being calculated as follows: torque percentage decrement score = [1 – (set 1 + set 2 + set 3 + set 4)/ (setbest × 4)] × 100. The percentage decrement score (40) takes into consideration all efforts (unlike the fatigue index score, which is influenced more by a particularly good first or bad effort) and was used to quantify the degree of performance loss by comparing the actual performance to an imagined ideal performance (i.e., one in which the best effort (setbest) would be replicated in each set (39)]. The raw root mean square (RMS) of the rectus femoris EMG signal produced during each contraction was calculated over the 150-ms period preceding the peak torque and averaged (five contractions) and normalized to the amplitude of the maximal M-wave value (see below) as an index of neural drive (RMS/M-wave ratio) for every repetition. Specifically, the M-wave values measured before and after a given exercise set were averaged to obtain one single M-wave value representative of each of the four exercise sets. For each neuromuscular test sequence, voluntary torque (MVC) and associated EMG activity (RMS) were recorded over the highest 1-s plateau preceding the superimposed twitch. The peak potentiated twitch torque (i.e., the highest value of twitch tension production) was located in the gluteal fold opposite the cathode. During the recruitment curve a passive isometric recruitment curve was drawn. Briefly, the stimulation intensity was progressively increased by 10 mA increments until plateaus occurred in twitch amplitude and compound muscle action potential (M-wave). Supramaximal stimulation was ensured by increasing the final intensity by 30% (mean current 95 ± 6 mA).

Near-infrared spectroscopy. Muscle and cerebral tissue oxygenation was monitored continuously throughout the exercise via near-infrared spectrometry (NIRS) (Oxymon MKIII; Artinis, The Netherlands). The theory, limitations and reliability of measurement obtained with this device during exercise are detailed elsewhere (77, 82). One NIRS emitter and detector pair was placed over the left prefrontal cortex, between Fp1 and F3 (international EEG 10–20 system). Spacing between optodes was fixed at 45 mm using a black, plastic spacer held in place via double-sided, stick disks and a black, tensioning headband to reduce the intrusion of extraneous light and the loss of transmitter NIRS from the field of investigation. A second emitter and detector pair was fixed on the distal part of the left vastus lateralis muscle bellies (−15 cm above the proximal border of the patella) using a black plastic spacer with an optode distance of 45 mm. Probes were secured to the skin using double-sided, stick disks and shielded from light using black elastic bandages. An indelible pen was used to mark the position of the optodes for subsequent visits. A modified form of the Beer-Lambert Law was used to calculate micromolar changes in tissue oxyhemoglobin (O2Hb) and deoxy-hemoglobin (HHb) across time using received optical densities from two continuous wavelengths of NIRS light (765 and 855 nm). An age-dependent differential optical path length factor for prefrontal cortex (69, 72) and a fixed differential path length of 3.83 for the vastus lateralis (25, 26) were used. The tissue saturation index (TSI, expressed in %), which reflects the dynamic balance between O2 supply and O2 consumption, was calculated as O2Hb/HHb × 100 for both the prefrontal cortex (cerebral TSI) and vastus lateralis (muscle TSI). NIRS data were acquired at 10 Hz and down-sampled to 1 Hz when transferring from the Oxymon MKIII to a personal computer. Data were averaged over each entire set (132 s) including both the exercise (∼7 s) and recovery time (15 s) of the six repetitions to obtain one value for each of the four sets. The post wash-in value was obtained over a 2-min period while the participant was instructed to sit as still as possible, to limit any extraneous thoughts, and to rest completely with their eyes closed.

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**Statistical analysis.** A one-way ANOVA with repeated measures for condition (NM, MH, SH) was used to test for differences in mean peak torque and RMS/M-wave ratio for the first repetition in set one. A one-way ANOVA with repeated measures for condition was also conducted on preexercise measures of voluntary and evoked force as well as %VA and M-wave. Because no significant difference was found between conditions for any of the above-listed variables, the mean peak torque and EMG RMS values for all subsequent repetitions were normalized to the value obtained during the first repetition of the first set for each condition, respectively. Subsequently, neuromuscular variables were expressed as a percentage of their respective preexercise values. A three-way ANOVA with repeated measures for condition (NM, MH, SH), sets (set 1, set 2, set 3, set 4) and repetitions (rep 1, rep 2, rep 3, rep 4, rep 5, rep 6) was used to test for within-group differences in the normalized mean peak torque and RMS/M-wave ratio. In addition, a two-way ANOVA with repeated measures for condition (NM, MH, SH) and time (pre wash-in and post wash-in) was used to test for within-group differences in resting heart rate and SpO2. A secondary two-way ANOVA with repeated measures for conditions (NM, MH, SH) and time (pre-exercise, post set 1, set 2, set 3, and set 4) was used to test for within-group differences in evoked and voluntary torque, percentage voluntary activation, cardio-respiratory and perceptual measures, as well as cerebral and muscle oxygenation. Mauchly’s test was used to assess for sphericity and in any cases of violation the Greenhouse-Geisser epsilon correction was used to adjust the degrees of freedom. When ANOVA revealed a significant main effect, pairwise comparisons were made using the Bonferroni method. Data are presented as means ± SE within the text and in all figures. Statistical analyses were performed using SPSS (version 19.0), and statistical significance was set at $P < 0.05$.

**RESULTS**

**Exercise performance and electromyography.** There was no significant effect ($P > 0.05$) of condition on the mean peak torque (NM 202.0 ± 10.8 N/m, MH 201.2 ± 9.6 N/m, SH 199.0 ± 9.0 N/m) or RMS/M-wave ratio (NM 0.082 ± 0.012, MH 0.080 ± 0.010, SH 0.079 ± 0.008) achieved during the first repetition in set 1. Compared with the first repetition, there was a significant condition and time interaction for the mean peak torque produced during each repetition within each set ($P < 0.05$) (Fig. 1). There was also a significant condition and time interaction on the total mean peak torque produced during each set ($P < 0.05$). Compared with NM, the mean peak torque was lower during set 3 in both MH and SH and during set 4 in SH only (all $P < 0.5$). A significant main effect for condition was also found on the percentage torque decrement score, which was greater in SH compared with NM only (NM 2.9 ± 1.3%, MH 5.2 ± 1.5%, SH, 10.3 ± 2.0%, $P < 0.05$).

Compared with the first repetition within each set, the mean RMS/M-wave ratio declined (main effect of time $P < 0.05$). Overall, from the first repetition in set 1 to the last repetition in set 4 the RMS/M-wave ratio was reduced by 5.4 ± 5.3%, 9.9 ± 4.7%, and 9.5 ± 5.2% in NM, MH, and SH, respectively, with no significant difference between conditions ($P > 0.05$). Compared with the mean RMS/M-wave ratio of the first set a main effect of time ($P < 0.05$) but not condition ($P > 0.05$) was present. Only when all conditions were compounded

[Fig. 1. Normalized peak torque ($A$) and normalized root mean square (RMS)/M-wave ratio ($B$) during each repetition. Values are means ± SE, $n = 14$. Data are presented as a percentage of the first repetition in set one for normoxia (FIO2 0.21), moderate hypoxia (FIO2 0.14), and severe hypoxia (FIO2 0.10). Interaction between time and condition on the mean peak torque produced during each repetition and during each set (both $P < 0.05$). Main effect of time on the RMS/M-wave ratio during each repetition and during each set (both $P < 0.05$).]
was the mean RMS/M-wave during set 4 significantly lower than during set 1 (P < 0.05).

Cardiorespiratory and perceptual parameters. Prior to entering the normobaric chamber, HR and SpO2 were not different among conditions [58 ± 2 beats per minute and 97 ± 0%, respectively; all conditions compounded, both P > 0.05]. Following the 10-min wash-in period, there was a hypoxia-severity dependent increase in HR and decrease in SpO2 (HR 60 ± 2, 69 ± 2, and 77 ± 3 bpm; SpO2 97 ± 0%, 90 ± 0%, and 78 ± 2%, for NM, MH, and SH respectively; both P < 0.05). During the exercise protocol there was no further change in SpO2 in any condition, whereas HR increased across the four sets of exercise in all conditions with no effect of condition (main effect of time, P < 0.05) (Fig. 2). RPE increased across sets in all trials (P < 0.05), but was not different among conditions (end-exercise value of 16.6 ± 0.6, all conditions compounded, P > 0.05).

Neuromuscular assessment responses. Preexercise values for all neuromuscular parameters did not differ (P > 0.05) between conditions (Table 1). Changes in MVC torque displayed a main effect of time (P < 0.05) but not condition (P > 0.05), with reductions in MVC torque from preexercise to post set 4 reaching 17.7 ± 1.6%, 19.3 ± 2.2%, and 26.5 ± 2.8% in NM, MH, and SH, respectively (Fig. 3A). Similarly, there was no main effect of condition on the reduction in VA from preexercise to post set 4 (3.7 ± 1.0%, all conditions combined, P < 0.05) (Fig. 3B). There was a significant interaction between time and condition for Qtw,pot (P < 0.05), with Qtw,pot being reduced following the first set in all conditions (P < 0.05) (Fig. 3C). The reduction in Qtw,pot following set 1 was different between SH and NM only (18.7 ± 3.0%, 21.4 ± 2.9%, and 25.8 ± 2.7% for NM, MH, and SH, respectively; P < 0.05). The decline in Qtw,pot was greater in SH compared with NM following every set (P < 0.05). Compared with preexercise, reductions in Qtw,pot at post set 4 reached 28.0 ± 3.2%, 32.1 ± 3.3%, and 41.3 ± 3.0% for NM, MH, and SH, respectively, with only SH being significantly different from NM (P < 0.05) (Fig. 3C). Preexercise M-wave, peak twitch torques at 20 Hz and 80 Hz, and 20/80 Hz ratio were no different across conditions (Table 1). M-wave remained unchanged across time in all conditions, whereas the 20/80 Hz ratio was reduced following the first set in all conditions (6.9 ± 1.3, all conditions compounded, P < 0.05) with no further change across time in any condition (post set 4, 8.6 ± 1.5, all conditions compounded, P > 0.05).

Muscle and cerebral tissue oxygenation. Following the wash-in period, cerebral TSI differed between conditions (89.3 ± 1.9%, 83.8 ± 2.6%, and 69.6 ± 3.5% for NM, MH, and SH respectively; all P < 0.05), but did not change thereafter (mean cerebral TSI during set 4 90.9 ± 2.3%, 80.8 ± 3.6%, and 65.1 ± 3.2% for NM, MH, and SH, respectively) (Fig. 2C). Following the wash-in period, there was no effect of condition on muscle TSI (P > 0.05) (Fig. 2D). Mean muscle TSI was reduced from post wash-in values during the first set of exercise in all conditions (main effect of time, P < 0.05), with no further changes thereafter. The mean muscle TSI during set 4 was reduced by 23.1 ± 2.7%, 21.6 ± 2.1%, and 27.8 ± 3.1% in NM, MH, and SH, respectively, with only SH being different from NM (P < 0.05).

DISCUSSION

Summary of main findings. We used hypoxia to explore the interplay between the development of peripheral fatigue and changes in central motor output and exercise capacity during maximal-intensity, isolated, dynamic leg extensions. As ex-
such, isolated exercise has been suggested to allow any given absolute workload to be carried out at a similar relative intensity under hypoxic conditions (28, 84). Nevertheless, the fact that the highest HR and the lowest muscle TSI occurred in MH and not SH is an interesting and unexpected finding, which indirectly illustrates that even when using a smaller muscle mass, convective oxygen transport may not be uniformly affected by hypoxia severity.

In the current study, despite less work being performed under hypoxic compared with normoxic conditions, we observed greater reductions in Q\textsubscript{tw,pot} following every set of MIIE during an exercise task that activated a smaller muscle mass than during whole-body exercise. Although previous research has reported greater reductions in Q\textsubscript{tw,pot} during exercise of equal work rates and equal durations (6, 10, 47, 65), our study is the first to show an exacerbated rate of peripheral fatigue development under SH compared with NM, despite a lower absolute exercise intensity in SH. Together, these results support the notion that hypoxia exacerbates the rate of peripheral fatigue development independently of changes in relative exercise intensity during submaximal isometric contractions.

| Table 1. Preexercise muscle function |
|-----------|-----|-----|
|           | Normoxia | Moderate Hypoxia | Severe Hypoxia |
| MVC torque, N/m | 290.6 ± 13.9 | 284.8 ± 13.8 | 284.9 ± 12.7 |
| VA, % | 98.2 ± 0.5 | 98.0 ± 0.4 | 97.8 ± 0.5 |
| Q\textsubscript{tw,pot}, N/m | 53.3 ± 3.5 | 53.1 ± 3.2 | 57.3 ± 2.9 |
| Db20Hz, N/m | 101.6 ± 5.3 | 98.9 ± 5.4 | 101.3 ± 4.6 |
| Db80Hz, N/m | 94.2 ± 4.7 | 93.2 ± 4.3 | 94.9 ± 4.0 |
| 20/80Hz | 1.07 ± 0.01 | 1.06 ± 0.01 | 1.07 ± 0.01 |
| M-wave, mV | 6.7 ± 3.2 | 6.6 ± 3.2 | 6.8 ± 3.5 |

MVC, maximal voluntary contraction torque; VA, voluntary activation; Q\textsubscript{tw,pot}, potentiated twitch torque; Db20Hz, torque associated with doublets at 20 Hz; Db80Hz, torque associated with doublets at 80 Hz; 20/80Hz, 20 Hz/80 Hz ratio; M-wave, compound muscle action potential amplitude measured after 10 min wash-in period to the experimental condition. Values are means ± SE, n = 14. Data are presented for normoxia (FiO\textsubscript{2} 0.21), moderate hypoxia (FiO\textsubscript{2} 0.14), and severe hypoxia (FiO\textsubscript{2} 0.10).
muscle deoxygenation and peripheral fatigue development following some form of conscious effort regulation (discussed below). In line with previous studies that have observed a reduction in EMG activity during MIIE (52, 62, 72) or changes in VA and reductions in muscle TSI (2) under NM conditions following the first set of MIIE (only 42 s of exercise time, 25% of total exercise time) highlights that peripheral fatigue occurs very early during repeated, maximal, isokinetic quadriceps contractions. Such early development of peripheral fatigue has recently been observed by Froyd et al. (35) who reported a ~43% reduction in evoked twitch force (~65% of the total end-exercise reduction) after only 48 s (20% of total exercise time) of repetitive isokinetic concentric knee extension-flexions at 300 deg/s during a 30,000 J time trial (35). These findings are unsurprising given the rapid quadriceps muscle deoxygenation that we and others have observed during whole-body MIIE (19) and submaximal isolated contractions (42, 47), and the subsequent accumulation of muscle metabolites that is known to occur during maximal intensity exercise of the same muscle group (21, 23, 37). In fact, a 15-fold increase in blood lactate concentration, Pi levels, and reductions in blood pH have been observed following only five maximal 6-s cycling sprints (30 s of total exercise time) (21, 37). Furthermore, we observed that in no condition did QM pot further decrease from post set 3 to post set 4, which supports previous research that has found no further reductions in peripheral fatigue during the second half of high-intensity, self-paced dynamic knee extensions (35) and repeated 6-min bouts of high-intensity cycling (29). This has been suggested as evidence that during self-paced, maximal exercise participants adopt a pace that can be kept relatively constant (35), indicating some form of conscious effort regulation (discussed below).

We also observed a similar kinetic in muscle TSI with reductions following the first set only in all conditions. In line with previous findings during whole-body MIIE (72), we found no differences in muscle TSI between NM and MH (simulated altitude of ~3,000 m) during repeated isolated contractions, while adding the observation of further reductions in muscle TSI under more severe hypoxic conditions. Therefore, despite the kinetic of muscle deoxygenation and peripheral fatigue development following the same trend in all conditions, the magnitude of those changes was exacerbated in SH.

Central motor output and voluntary activation. Traditionally, fatigue during short-duration, maximal intensity exercise has been attributed primarily to peripheral factors such as metabolic perturbations and muscle damage (17, 20, 35, 39, 68). Our finding of an ~8% decrease in the RMS/M-wave ratio (all conditions compounded), together with a modest (3.7%, all conditions compounded) but significant decline in voluntary activation (twitch interpolation technique), indicates that during our test protocol central motor output was reduced, which may have also contributed to the inability to maintain the initial torque during subsequent contractions. These findings are in line with previous studies that have observed a reduction in EMG activity during MIIE (52, 62, 72) or changes in VA following repeated isolated contractions (42) and support the presence of a central origin of fatigue and the suggestion that reduced neural activation of the contracting muscles contributes to the performance loss during both whole-body and isolated MIIE (84). Several factors have been suggested to impair central motor output under both normoxic and hypoxic conditions and are discussed below.

Central regulation of peripheral fatigue development. In contrast to our hypothesis, the central motor output downregulation that we observed (~8% decrease in RMS/M-wave, all conditions compounded) did not limit the development of peripheral muscle fatigue to a similar level across the various conditions of inspired oxygen fraction during isolated MIIE. Our hypothesis was based on the interpretation of the findings that manipulating arterial oxygen saturation (via changes in the Fo2) resulted in altered muscle activation, power output, and time-trial performance, despite similar end-exercise levels of potentiated twitch torque (peripheral fatigue) assessed between 2 and 2.5 min following exercise (6, 10). These authors concluded that the CNS may attenuate the level of muscle activation to regulate and limit the development of peripheral locomotor muscle fatigue via feedback from small-diameter III/IV-mediated muscle afferents (8, 9, 13, 30). Subsequent investigations incorporating open-loop designs [submaximal exercise of undetermined duration (75)] consisting of repeated submaximal isometric contractions performed to task failure, have supported a critical threshold of peripheral fatigue (42, 54). However, in one of those studies the interpretation of a critical threshold of muscle fatigue was based on the finding of similar decrements in MVC, whereas the reductions in QM pot were not identical across the four hypoxic conditions (42). Furthermore, in the study by Millet et al. (54) despite a similar level of peripheral fatigue following intermittent isometric knee extensions performed until exhaustion, the end-exercise level of quadriceps RMS activity was higher in the hypoxic condition. This would indicate a higher level of motor drive, which does not support the hypothesis that central motor output is regulated to limit excessive peripheral fatigue development during the respective exercise model. The authors acknowledged that a major influence of hypoxia on the CNS would have resulted in a lower maximal EMG in hypoxic conditions, which was not the case. As such, these results support our observation that peripheral fatigue development was not independently regulated via alterations in central motor output.

It has recently been suggested that power output is adjusted during maximal repeated cycling sprints to restrain peripheral fatigue development to a constant threshold (46). This interpretation was based on the observation of similar end-exercise...
levels of peripheral fatigue following two repeated sprint trials (10 × 10-s sprints interspersed with 30 s of recovery), despite varying levels of preexisting muscle fatigue obtained via electrically induced quadriceps stimulations. Although supporting the role that peripheral fatigue may play in limiting maximal exercise performance, our finding of varying levels of end-exercise peripheral fatigue following maximal-intensity exercise under different hypoxic conditions challenges the interpretation that the level of peripheral fatigue per se is regulated below an individual critical threshold (46). In fact, it has previously been shown that the end-exercise level of peripheral fatigue was significantly less following an exhaustive, single leg, knee extensor exercise immediately after the same exercise task was completed using the contralateral leg (12), highlighting that the absolute amount of peripheral muscle fatigue is not a variable that is critically regulated.

Although we did not measure respiratory muscle fatigue, it has previously been shown that exercise involving a smaller muscle mass avoids the hypoxia-induced demands on convective O2 transport to the active muscles and the subsequent exacerbation of respiratory muscle fatigue that has been shown to lead to an earlier conscious decision to stop exercise during whole-body exercise (49, 50). In fact, it was recently observed that the level of end-exercise peripheral fatigue is greater following exhaustive small vs. large muscle mass exercise to task failure at the same percentage of the modality-specific maximal workload despite the reported RPE being no different across conditions (66). These results suggest that when the source of afferent feedback is confined to a smaller exercising muscle mass, a greater local accumulation of metabolic by-products and concomitant development of peripheral fatigue may be reached before an equal magnitude of ensemble afferent feedback is achieved (66). Together with the findings of the present study, these results indicate that adjustments in central motor output and muscle activation do not appear to be regulated to restrict peripheral fatigue below a critical threshold. Rather, these results suggest that the sum of sensory inputs into the CNS may act to curtail central motor output and muscle activation in accordance with an individual sensory tolerance limit (13, 38), which may be consciously regulated in accordance with various psychological factors [i.e., level of motivation, previous experience of the task, and knowledge of the task duration remaining (51)]. In the present study, it therefore appears likely that the sum of sensory inputs that subjects experienced during the severe hypoxic condition was insufficient to exacerbate the decline in central motor output and thereby attenuate the development of peripheral fatigue to a similar level.

Although a reduction in neural drive as a consequence of an anticipatory preexercise pacing strategy (14) has been shown to be less likely during maximal short-duration efforts of less than 15 s (85), it also remains possible that despite having previous experience/memory of the task and being instructed to give a maximal, all-out effort, participants adopted some form of anticipatory pacing strategy as a consequence of being blinded to the environmental conditions (FiO2). In fact, an anticipatory reduction in muscle activation was recently observed during a bout of repeated cycling sprints when participants were deceived about the number of sprints remaining (18). As such, an anticipatory downregulation of central motor drive may have also occurred in the current study to delay the occurrence of fatigue and/or intolerable discomfort, and subsequently optimize performance, because the rate of development of physiological and perceptual responses during the task were unknown.

Cerebral oxygenation and arterial oxygen saturation. Mounting evidence also indicates that reductions in cerebral oxygenation may lead to direct affects on the CNS, altered central motor output and, ultimately, impaired exercise performance during acute severe hypoxia (8, 20, 72, 84). This direct effect of hypoxia on the CNS is based on at least three observations from the literature: 1) the ability to sustain the target workload at task failure immediately following a shift from severe hypoxia to hyperoxia (11, 28, 48, 78, 80) or normoxia (27); 2) exercise-induced reductions in cortical voluntary activation during exercise in severe hypoxia (41, 42); and 3) reductions in exercise performance in severe hypoxia during isolated contractions despite metabolic conditions being kept identical through the use of an occlusion cuff (54, 55).

In the present study, resting cerebral oxygenation was reduced under increasing levels of hypoxia severity, as previously observed (4, 59, 67). However, reductions in cerebral oxygenation did not appear to lead to any hypoxia-severity-dependent effects on central motor output or voluntary activation (Fig. 3B), which may be explained in part by a combination of factors, including the exercise mode and duration used, without considering limitations involved with the voluntary activation technique (38). In fact, reductions in cerebral oxygenation under hypoxic conditions have been shown to be attenuated or even alleviated during exercise of isolated muscles, in contrast to whole-body exercise where cerebral deoxygenation is accentuated (84). In the current investigation, we failed to observe any exercise-induced arterial desaturation, which has previously been shown to occur in parallel with a greater increase in cerebral deoxygenation during whole-body cycling sprints of comparable duration and performed at a lower simulated altitude of 3,700 m (72). These differences among exercise modes have been suggested to occur as a result of an elevated cerebral metabolic rate and O2 consumption during whole-body exercise, and may explain why central fatigue was less during our model compared with whole-body exercise (57, 69, 84). Our findings are in line with those of Goodall et al. (42) who recently reported no difference in the reduction of peripheral voluntary activation following intermittent isometric quadriceps contractions performed under varying degrees of hypoxia severity. Together, these results highlight the role of the exercising muscle mass in the development of cerebral oxygenation-mediated declines in central motor output.

Limitations. Despite paired stimuli (Db) being suggested as an appropriate method for calculating VA due to a high signal-to-noise ratio (3, 58), it is likely that our superimposed Db amplitude (and therefore VA values) may have been increased with greater stimulation frequencies or stimulation trains (53). However, with improvements in the signal-to-noise ratio when using increased numbers of pulse stimuli potentially countered by cofounders such as greater antidromic activation of motoneurons and Renshaw cells (34, 38), it is likely that the most important factor in determining VA is the resolution of the superimposed torque, which was not a concern in the current investigation. In fact, while obviously dependent on the task being performed, it is
interesting to observe that the magnitude of reductions in VA at exercise cessation (3.7 ± 1.0%) are not only in an acceptable agreement with those classically reported using higher stimulation frequencies or pulse trains for exercise of similar nature (44), but also were sensitive enough to display a significant main effect of time. Additionally, it is possible that the statistical power of the current study may have been insufficient to identify differences in some other variables, such as MVC torque due to large intra- or interday variability (22), or a lower reliability in fatigued conditions (61). Of note, our finding of significant differences across conditions in the end-exercise reduction of Q_{tw,pot} despite no main effect of condition in the reduction of MVC torque has also previously been shown by others (42). Finally, muscle recruitment was estimated from one muscle only. Additional EMG recordings from antagonist muscles may have provided more insight into the effect of hypoxia on alterations in recruitment strategies during repeated, short-duration, all-out efforts in a closed-loop design. However, it is unlikely that any of these above-mentioned points would have altered the main findings of the current investigation or our main interpretation that peripheral fatigue is not a critical variable that is independently regulated via adjustments in central motor output.

Conclusion. In conclusion, both central and peripheral adjustments occur during maximal-intensity, intermittent leg extensions, with severe hypoxia exacerbating both peripheral fatigue development and impairments in exercise capacity. Through the use of closed-loop, repeated, maximal leg extensions, with almost immediate assessments of neuromuscular function throughout, we failed to support the proposal that peripheral fatigue is independently regulated via adjustments in central motor output.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: R.J.C., D.J.B., F.B., and O.G. conception and design of research; R.J.C. and O.G. performed experiments; R.J.C., D.J.B., F.B., and O.G. analyzed data; R.J.C., D.J.B., F.B., and O.G. interpreted results of experiments; R.J.C. and O.G. prepared figures; R.J.C., D.J.B., F.B., and O.G. drafted manuscript; R.J.C., D.J.B., F.B., and O.G. edited and revised manuscript; R.J.C., D.J.B., F.B., and O.G. approved final version of manuscript.

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