Effects of arterial oxygen content on peripheral locomotor muscle fatigue

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Amann, Markus, Lee M. Romer, David F. Pegelow, Anthony J. Jacques, C. Joel Hess, and Jerome A. Dempsey. Effects of arterial oxygen content on peripheral locomotor muscle fatigue. J Appl Physiol 101: 119–127, 2006. First published February 23, 2006; doi:10.1152/japplphysiol.01596.2005.—The effect of arterial O2 content (CaO2) on quadriceps fatigue was assessed in healthy, trained male athletes. On separate days, eight participants completed three constant-workload trials on a bicycle ergometer at fixed workloads (314 ± 13 W). The first trial was performed while the subjects breathed a hypoxic gas mixture [inspired O2 fraction (FiO2) = 0.15, Hb saturation = 81.6%; CaO2 = 18.2 ml O2/dl blood; Hypo] until exhaustion (4.5 ± 0.4 min). The remaining two trials were randomized and time matched with Hypo. The second and third trials were performed while the subjects breathed a normoxic (FiO2 = 0.21, Hb saturation = 95.0%; CaO2 = 21.3 ml O2/dl blood; Norm) and a hyperoxic (FiO2 = 1.0, Hb saturation = 100%, CaO2 = 23.8 ml O2/dl blood; Hyper) gas mixture, respectively. Quadriceps muscle fatigue was assessed via magnetic femoral nerve stimulation (1–100 Hz) before and 2.5 min after exercise. Myoelectrical activity of the vastus lateralis was obtained from surface electrodes throughout exercise. Immediately after exercise, the mean force response across 1–100 Hz decreased from preexercise values (P < 0.01) by −26 ± 2, −17 ± 2, and −13 ± 2% for Hypo, Norm, and Hyper, respectively; each of the decrements differed significantly (P < 0.05). Integrated electromyogram increased significantly throughout exercise (P < 0.01) by 23 ± 3, 10 ± 1, and 6 ± 1% for Hypo, Norm, and Hyper, respectively; each of the increments differed significantly (P < 0.05). Mean power frequency fell more (P < 0.05) during Hypo (−15 ± 2%); the difference between Norm (−7 ± 1%) and Hyper (−6 ± 1%) was not significant (P = 0.32). We conclude that ΔCaO2 during strenuous systemic exercise at equal workloads and durations affects the rate of locomotor muscle fatigue development.

Hypo; hypoxia; hemoglobin saturation; magnetic femoral nerve stimulation

ACUTE EFFECTS OF ARTERIAL O2 content (CaO2) on exercise performance have been studied extensively. It is generally accepted that a reduction in CaO2 impairs and an elevation in CaO2 improves whole body exercise performance (30). These observations in health have been confirmed by studies in patients with chronic obstructive pulmonary disease (32, 41, 55) and anemia (56). However, although the positive effects of elevated CaO2 on endurance performance are widely accepted, controversy exists regarding its effects on peripheral locomotor muscle fatigue induced by systemic exercise. Some reports do not support an exaggerating effect of low CaO2 on muscle fatigue associated with the same relative (36) or absolute (42, 52) workload at equal exercise durations. Other investigations have demonstrated a clear aggravation of locomotor muscle fatigue, as assessed via electromyogram (EMG) recording, induced by prolonged exercise with low CaO2 (59, 60). We recently demonstrated that even the prevention of exercise-induced arterial hypoxemia during heavy sustained exercise to exhaustion (17) significantly attenuated quadriceps muscle fatigue assessed via pre- and postexercise femoral nerve stimulation (51). We have now extended this work to determine the effects of a wide range of CaO2 on locomotor muscle fatigue during exercise. In addition, we have also adopted a method capable of assessing fatigue during exercise in addition to the established measurement of force loss from pre- to postexercise in response to supramaximal magnetic nerve stimulation.

The intention of this investigation was to test the hypothesis that peripheral locomotor muscle fatigue is highly sensitive to changing CaO2 over a wide range during systemic exercise. Our hypothesis was uniquely supported by demonstrating CaO2-dependent differences in (1) the development of peripheral fatigue during exercise and (2) the levels of excitation-contraction coupling (ECC) failure immediately after exercise.

METHODS

Subjects

Eight healthy, trained male cyclists [mean ± SE: 22.5 ± 1.7 yr old, 69.8 ± 2.8 kg body wt, 173.9 ± 3.0 cm, 63.5 ± 1.3 ml·kg−1·min−1 maximal O2 consumption (VO2max)] volunteered to participate in the project. All subjects had normal resting pulmonary functions. Written informed consent was obtained from each participant. The protocol was approved by the institution’s human subjects committee.

Physiological Responses to Exercise

Ventilation and pulmonary gas exchange were measured breath-by-breath at rest and throughout exercise via an open-circuit system (34). Heart rate was measured from R-R interval of an electrocardiogram using a three-lead arrangement. Ratings of perceived exertion (dyspnea and limb discomfort) were obtained at the end of each exercise trial using Borg’s modified CR10 scale (10). Blood (Hb) O2 saturation (SPO2) was estimated using a pulse oximeter (Nellcor OxiMax, Pleasanton, CA), with optodes placed on the forehead. The output of the pulse oximeter (SPO2) has been shown to be in good agreement (intraclass correlation coefficient = 0.88) with HbO2 saturation based on arterial blood analysis (SaO2) (51). CaO2 calculation was based on the subject’s Hb concentration obtained from a radial artery catheter during a recent investigation [unpublished data; inspired O2 fraction (FiO2) = 0.21] at similar workloads and times. Arterial PO2 (PaO2) was estimated by subtracting the alveolar-arterial PO2 difference (adopted from a recent investigation) from end-tidal PO2, and HbO2 saturation was estimated from SPO2.

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Contractile Function, Myoelectrical Activity, and Membrane Excitability

EMG acquisition and analysis. Quadriceps EMG was recorded from the right vastus lateralis (VL), vastus medialis (VM), and rectus femoris (RF) via monitoring electrodes attached with full-surface solid adhesive hydrogel (Kendall H59P, Mansfield, MA), with on-site amplification. Electrodes were placed in a bipolar electrode configuration on the respective belly of the muscle. The active electrode was placed over the motor point, located at the middle of the muscle belly. The recording electrode was moved along the muscle until a good configuration, confirmed by a “maximal” M-wave shape, was achieved. The reference electrode was placed over an electrically neutral site (epicondyle of femur). The resulting interelectrode distances were smaller for VL and VM (60–80 mm) and wider for RF (up to 100 mm). Proper electrode configuration was checked before the beginning of every experiment. The position of the EMG electrodes was marked with indelible ink to ensure that they were placed in the same location at subsequent visits. To ensure low levels of movement artifacts, electrode cables were fastened to the subjects’ quadriceps with medical adhesive tape and wrapped in elastic bandage. The surface electrodes were used to record combined neural and muscular EMG: 1) magnetically evoked compound muscle action potentials (M waves) for VL, VM, and RF to evaluate changes in M-wave properties and 2) EMG continuously throughout all constant-workload cycling trials for VL to manifest fatigue. Membrane excitability was assessed before and immediately after exercise for VL, VM, and RF using M-wave properties evoked by supramaximal magnetic stimuli. The characteristics measured included peak amplitude, duration, and conduction time (14, 53). The duration was defined as the time from baseline to baseline from the beginning to the end of the biphasic M wave, where the beginning is defined as a positive deflection 2 SDs above baseline harmonic mean and the end as a return to baseline. The conduction time was defined as the time between the stimulus artifact and peak. Raw EMG signals from VL corresponding to each muscle contraction during the constant-workload trials and the pre- and postexercise maximal voluntary contraction (MVC) maneuvers were recorded for later analysis. The EMG signal was amplified and filtered by a Butterworth band-pass filter (model BMA-830, CWE, Ardmore, PA) with a low-pass cutoff frequency of 10 Hz and a high-pass cutoff frequency of 1 kHz. The slope of the filters was −6 dB/octave. The filtered EMG signal was sampled at 2 kHz by a 16-bit analog-to-digital converter (model PCI-MIO-16XE-50, National Instruments, Austin, TX) with custom software (Labview 6.0, National Instruments). A computer algorithm identified the onset of activity where the rectified EMG signal deviated by >2 SDs above the baseline for ≥100 ms. Each EMG burst was visibly inspected to verify the timing identified by the computer. For data analysis, the integral of each burst [integrated EMG (iEMG)] was calculated using the following formula

\[
iEMG[m(t)] = \int_{0}^{t} m(t)\, dt
\]

where \(m\) is the raw EMG signal.

A 1,024-point fast Fourier transform was used to compute a power spectrum periodogram. The mean power frequency (MPF) was calculated using the following formula

\[
MPF = \frac{\int_{0}^{\infty} f S_{m}(f)\, df}{\int_{0}^{\infty} S_{m}(f)\, df}
\]

where \(S_{m}(f)\) is the power density spectrum of the EMG signal.

Magnetic stimulation. Magnetic stimulation was carried out as described in detail previously (51). Briefly, subjects lay supine on a table, with the right thigh resting in a preformed holder and the knee joint angle set at 1.57 rad (90°) of flexion. Two magnetic stimulators (model 200, Magstim, Wales, UK; www.magstim.com) were used to stimulate the femoral nerve (39, 49), and the evoked quadriceps twitch force (Qtw) was obtained from a load cell (model SM 1000, Interface, Scottsdale, AZ). We used single (1-Hz) and paired stimuli (interstimulus intervals = 100 ms (−10 Hz), 50 ms (−20 Hz), and 10 ms (−100 Hz)) to discriminate between low- and high-frequency fatigue (48, 64). To determine whether nerve stimulation was supramaximal, three single twitches were obtained every 30 s at 50, 60, 70, 80, 85, 90, 95, and 100% of maximal stimulator power output at the beginning of every experiment. A near plateau in baseline Qtw and M-wave amplitudes with increasing stimulus intensities was observed in every subject, indicating maximal depolarization of the femoral nerve (Fig. 1). Twitch force at 100% of maximal stimulator power output measured at the beginning of the progressive increase in power output was not different from that at the end, indicating that the incremental protocol did not elicit twitch potentiation. After a 20-min rest period, six MVCs of the right quadriceps, separated by 30 s, were performed for 5 s each. To obtain potentiated twitch force, Qtw in response to a single twitch was measured 5 s after each MVC. Activation of the quadriceps during the MVC was assessed using a superimposed twitch technique (45, 58). Briefly, the force produced during a superimposed single twitch on the MVC was compared with the force produced by the potentiated single twitch delivered 5 s later (58). A correction was applied to the superimposed twitch, because it did not always occur at maximal voluntary force (58). Next, paired stimuli (100, 50, and 10 Hz) were repeated four times for each frequency, followed by eight single stimuli (1 Hz). The stimulations were each separated by 30 s. The entire assessment procedure took 15 min to complete and was performed before exercise (~30 min) while the

Fig. 1. Quadriceps twitch force (Qtw, A) and M-wave amplitudes (B) as a direct response to magnetic stimulation of the femoral nerve by application of single twitches (1 Hz) at increasing stimulator power settings [inspired O2 fraction (FIO2) = 0.21]. Incremental protocol was applied after a 10-min rest period and completed 15 min before preexercise assessment of neuromuscular function. EMG activity was recorded from 3 pairs of surface electrodes (rectus femoris, vastus medialis, and vastus lateralis), and M-wave amplitudes were analyzed using a customized software program. %max, Percentage of maximum.
subjects breathed room air and again started 2.5 min after exercise while the subjects continued to breathe the gas that was administered during the exercise bout. Qtw in response to each stimulus was analyzed. To compare force development characteristics, contraction time (CT), maximal rate of force development (MRFD), one-half relaxation time (RT0.5), and maximal relaxation rate (MRR) were analyzed for all single twitches (40, 53). Reliability of magnetic stimulation. To test for between-day reliability, the subjects repeated the magnetic stimulation protocol at rest on separate visits to the laboratory. For within-day reproducibility, the subjects were removed from the testing apparatus after baseline measurements of muscle function had been obtained and rested in a chair for 30 min without contracting the quadriceps; then they were attached to the testing apparatus, and measurements of quadriceps muscle function were repeated. There was no systematic bias in the baseline measurements within or between days. Mean within-day within-subject coefficients of variation for Qtw across all frequencies were 1.9 ± 0.5% (range 0.1–7.6) and 3.9 ± 0.7% (range 0.0–8.8) for MVC and 1.8 ± 0.7% (range 0.2–3.9) for voluntary muscle activation. Mean between-day within-subject coefficients of variation for Qtw across all frequencies were 4.2 ± 0.9% (range 2.1–6.3) and 6.0 ± 0.6% (range 3.9–7.1) for MVC and 1.9 ± 0.3% (range 1.0–2.9) for voluntary muscle activation. The reliability of the magnetic stimulation technique observed in this study was very similar to an earlier investigation in our laboratory (51).

Protocol

At a preliminary visit to the laboratory, the subjects were familiarized thoroughly with the procedures used to assess quadriceps muscle function and performed a maximal incremental exercise test [FIO2 = 0.21, workload = 20 W + 25 W/min (3)] on an electrically braked cycle ergometer (Velotron Elite, Racer Mate, Seattle, WA) for the determination of peak power output. On later occasions, blinded to the respective FIO2, subjects completed three constant-workload trials at the same time of the day separated by ≥48 h. Mean power output and pedal cadence from previously completed 5-km bicycle time trials (FIO2 = 0.21; data not shown) were adopted to establish the subject’s individual fixed workload for the constant-workload trials (313.8 ± 12.9 W, equals 82.5 ± 1.7% peak power output in normoxia, 105.7 ± 2.5 rpm). The first trial was performed while the subject breathed a humidified hypoxic gas mixture (FIO2 = 0.15, Hypo) to the limit of tolerance, and exercise was terminated when pedal cadence fell below 95% of target pedal frequency. Constant visual and vocal feedback was given to the subjects to avoid variations in pedal cadence. The recording period started after the target pedal cadence was reached (<6 s). Neuromuscular function was assessed before and at 2.5 min after exercise. EMG activity of VL was recorded during all trials. All cycling trials were preceded by a 10-min individualized warm-up at 1.5 W/kg body wt.

Statistical Analysis

One-way within-subjects ANOVA was used to determine the effects of the different CaO2. If ANOVA yielded a significant result, follow-up pairwise comparisons using Holm’s sequential Bonferroni procedure were conducted. Values are means ± SE. The α-level was set at 0.05 a priori.

RESULTS

CaO2 and Exercise Duration

Applying various FIO2 levels during exercise affected two determinants of CaO2: the arterial Hb saturation and the amount of dissolved O2. The contribution of PaO2 to total O2 content at end exercise was negligible when FIO2 = 0.21 and FIO2 = 0.15 were compared (~0.08 ml/dl); the main loss came from Hb desaturation (~3 ml/dl). However, with FIO2 = 1.0 vs. FIO2 = 0.21, both determinants contributed about equally to CaO2: ~1.1 ml/dl was gained by maximizing Hb saturation, and ~1.5 ml/dl was gained by the increase in PaO2.

The exercise time in all three conditions was 269.7 ± 21.9 s. Norm and Hyper were terminated when the subjects reached their individual performance time to exhaustion achieved in Hypo. End-tidal PaO2, SpO2, and CaO2 averaged 108 ± 1 Torr, 95.0 ± 0.6%, and 21.3 ± 0.6 ml O2/dl blood in Norm; 82 ± 2 Torr, 81.6 ± 1.9%, and 18.2 ± 0.6 ml O2/dl blood in Hypo; and 622 ± 5 Torr, 100%, and 23.8 ± 0.7 ml O2/dl blood in Hyper.

Magnetic Nerve Stimulation

M waves. As a measure for membrane excitability we examined pre- vs. postexercise M-wave characteristics in conjunction with the muscle mechanical properties for VL, VM, and RF in all conditions. Although there was a trend toward an increased M-wave amplitude, increased M-wave duration, and decreased conduction time after exercise, none of these changes were significant. These trends were similar for various levels of FIO2, suggesting that M-wave properties were independent of CaO2.

Contractile function. The effects of isometric constant-workload exercise with various levels of FIO2 on contractile function are summarized in Table 1 and Fig. 2. Qtw. For all levels of FIO2, postexercise Qtw across the various stimulation frequencies decreased significantly from preexercise baseline (Fig. 2). Force output (mean of 4 frequencies) was decreased by 34.6 ± 9.6 N in Norm, 54.4 ± 13.8 N in Hypo, and 27.5 ± 7.8 N in Hyper 2.5 min after exercise. A predominant low-frequency fatigue was indicated by the proportionately greater loss in Qtw (∆Qtw) associated with low- than with high-frequency stimulation.

The reductions in Qtw were significantly greater in Hypo than in Norm and greater in Norm than in Hyper for 1 Hz potentiated, 1 Hz, 10 Hz, and 50 Hz. For example, with the 1-Hz potentiated twitch (Table 1, Fig. 2), Hypo caused on average 59% greater reduction in force than Norm, whereas Norm caused a 14% greater reduction in twitch force than Hyper. For 100 Hz, ∆Qtw was significantly greater in Hypo than in Norm and Hyper, inasmuch as the loss in twitch force was not significant between Norm and Hyper (Table 1).

Within-twitch measurements. Significant differences in twitch relaxation properties were found for 1) RT0.5, which was increased more after Hypo than after Norm and more after Norm than after Hyper (P < 0.05), and 2) MRR was reduced more after Hypo than after Norm and more after Norm than after Hyper (P < 0.05). The prolongation in RT0.5 after Hyper was not significant from preexercise values (Table 1). MRFD was significantly more reduced after Hyper than after Norm and more reduced after Norm than after Hyper. CT was not significantly altered from preexercise baseline after Norm and Hyper, and various reductions were not different between CaO2 conditions.

MVC force and voluntary muscle activation. Peak force during the 5-s MVC maneuvers was significantly decreased from baseline after Hypo (11 ± 2%, P < 0.01), whereas Norm and Hyper did not result in a significant reduction (P = 0.67
and \( P = 0.93 \), respectively). Voluntary quadriceps activation, as assessed via the superimposed twitch technique, averaged 96–98% and was not affected by the preceding exercise at any \( \text{CaO}_2 \) (Table 1, Fig. 3).

**EMG Parameters**

\( iEMG \). \( iEMG \) of VL rose significantly from the 1st min to the final minute of exercise at all \( \text{CaO}_2 \),\( P < 0.01 \); Table 1, Fig. 4). The exercise-induced increase in \( iEMG \) was 13.0 ± 2.7% greater in Hypo than in Norm (\( P < 0.01 \)) and 4.1 ± 1.4% greater in Norm than in Hyper (\( P < 0.05 \)).

\( MPF \). The continuous increase in \( iEMG \) was associated with a significant decrease in \( MPF \) from the 1st min to the termination of exercise at all \( \text{CaO}_2 \), (Table 1, Fig. 4). At the termination of exercise, \( MPF \) had fallen significantly more during Hypo than during Norm and Hyper, but Norm and Hyper did not differ (\( P = 0.32 \)).

**Relation of the Two Fatigue Assessment Methods**

Figure 5 contrasts the group mean reductions in force output from pre- to postexercise with the corresponding changes in myoelectrical activity during the exercise. In general, the results suggest that if a certain level of peripheral quadriceps fatigue is detected by reductions in force output in response to magnetic nerve stimulation before vs. after exercise, this reduction is also reflected to a significant extent in the magnitude of the increase in \( iEMG \) and decrease in \( MPF \) throughout the exercise.

**Ventilation and Effort Perceptions**

The effects of \( \text{FiO}_2 \) on physiological responses during the final minute of constant-load exercise are represented in Table 2. In Norm, \( \text{O}_2 \) uptake (\( \text{VO}_2 \)) consistently rose from the beginning of exercise and reached 93.5 ± 3.6% \( \text{VO}_2 \max \) at the end of the trial. Starting at the 2nd min, \( \text{VO}_2 \) was consistently ~10% higher in Norm than in Hypo (\( P < 0.05 \)). Minute ventilation (\( \text{VE} \)) was significantly higher during Hypo than during Norm (26 ± 7%) and Hyper (36 ± 14%). Both ratings of perceived exertion (limb and dyspnea) indicate a significantly lower perception of effort at end exercise in Hyper than in Norm and in Norm than in Hypo.

**DISCUSSION**

The purpose of this study was to determine the consequences of \( \text{CaO}_2 \), as affected by \( \text{FiO}_2 \), \( \text{SaO}_2 \), and \( \text{PaO}_2 \), on peripheral quadriceps fatigue induced by high-intensity cycling exercise in healthy humans. We confirmed the effect of \( \text{CaO}_2 \) across various exercise conditions that were identical in work rate and duration. We assessed exercise-induced quadriceps fatigue by the reduction in force output in response to magnetic nerve stimulation before vs. after exercise and by changes in myoelectrical activity during the exercise. Reducing \( \text{CaO}_2 \) by ~15% from Norm to Hypo exaggerated the exercise-induced reduction in \( Q_{tw} \) by >50% and resulted in an over twofold greater rise in \( iEMG \) and concomitant twofold greater drop in \( MPF \) during exercise. Increasing \( \text{CaO}_2 \) by 12% from Norm to Hyper reduced \( \Delta Q_{tw} \) by ~20% and attenuated the raise in \( iEMG \) by ~40% and the fall in \( MPF \) by ~19%. The ~30% higher \( \text{CaO}_2 \) during Hyper than during Hypo attenuated the reduction in \( Q_{tw} \) by about one-half and induced the rise in \( iEMG \) from the 1st min to the last minute of exercise by about eightfold. The results indicate a high sensitivity of peripheral locomotor muscle fatigue to \( \text{CaO}_2 \) during whole body high-intensity exercise conducted at identical constant workload and duration.

**Technical Considerations**

Underestimation of total fatigue. Our stimulation technique to detect quadriceps fatigue assumed that the motor nerve input to the quadriceps, via the femoral nerve, was the same and supramaximal before and after exercise for each of the com-
investigation. In separate isolated quadriceps experiments (data unpublished), we used repeated isometric contractions to induce the same level of peripheral quadriceps fatigue on three different days. Before and during exercise, subjects inhaled room air or a hypoxic (FiO₂ = 0.105) or hyperoxic (FiO₂ = 1.0) gas mixture. The participants continued to breathe the respective gas mixture during the recovery period in which the postexercise fatigue assessment (using a similar protocol) was conducted. There was no effect of various CaO₂ on the change in Qtw in the immediate postexercise recovery (~10 min). In addition, in our previous study (51), we also showed a highly significant effect on exercise-induced quadriceps fatigue of small changes in CaO₂ (SaO₂ = 98 vs. 92%), even when both postexercise recovery periods were in normoxia.

M-wave characteristics. Our results show that membrane excitability of the peripheral motor nerve was not significantly altered by the exercise under any condition, as indicated by the consistent magnitude of the evoked potentials before vs. after exercise. This observation confirms that the measured changes in Qtw are mainly due to changes within the quadriceps and that peripheral failure of electrical transmission might be excluded. Additionally, the observation that changes in M-wave properties across trials were independent of the FiO₂ during recovery suggests that CaO₂, per se, does not affect neuromuscular transmission and/or muscle membrane propagation characteristics.

Surface EMG. Surface EMG activity is modified during muscular fatigue, which is reflected in variations in the time (iEMG) and frequency domain (MPF) (22). These variables have frequently been used as indicators for fatigue during static (7, 14) as well as dynamic (11, 25, 27, 32, 54, 60) exercise. A spectral shift toward lower frequencies and an increase in signal amplitude have generally been accepted as a sign of muscle fatigue (6), although this method has its shortcomings (see blow).

Although we found reasonable agreement between fatigue assessed by EMG variables and magnetic nerve stimulation, we believe that the use of surface EMG as a sole determinant of fatigue is questionable. 1) EMG variables cannot be used as indicators of contractile fatigue, because ECC failure as a potential contributor cannot be detected (22). However, meta-

Fig. 3. Effects of various FiO₂ on maximal voluntary contraction (MVC) force of quadriceps muscle. Gray bars, actual force voluntarily generated by the quadriceps during preexercise (Pre) and postexercise (Post) MVC maneuvers given the measured levels of muscle activation (in parentheses) using a superimposed twitch technique (45); open bars, deficit between voluntarily activated and predicted maximal force with assumption of 100% muscle activation. Reduction in MVC force was not significant after normoxia or hyperoxia trial (P = 0.67 and P = 0.93, respectively); hypoxia trial resulted in an 11% decrease of MVC force immediately after exercise (P < 0.01). There were no significant between-day differences in preexercise muscle activation (P = 0.61). Reductions in postexercise voluntary muscle activation were not significant (P > 0.1). *P < 0.01 vs. preexercise.
bolic fatigue is more rapid in onset and recovery than ECC fatigue (23) and, thus, might have played a key role in the detection of fatigue during exercise, which is reflected by EMG variables. 2) A number of intrinsic factors other than muscle fatigue have to be considered for alterations in surface EMG (5), confounding its use as a measure of neural drive to the muscle. It is, for example, crucial to acknowledge significant filtering effects on the EMG that are related to the thickness of the subcutaneous tissues and overrepresent motor units located near the recording electrodes. Additionally, amplitude cancellation can attenuate increases in motor unit activity measured by surface EMG. This insensitivity of the surface EMG technique might underestimate increases in neural drive/motor unit activity (37). However, normalized EMG has been shown to increase reliability compared with absolute EMG values (65). Accordingly, we view the usefulness of our EMG findings in the present study as confirmation from an indirect index of fatigue development during exercise, which is reflected by EMG variables.

Within-twitch responses. A valid method to identify perturbations in ECC as a major factor of fatigue is the use of the force-frequency relation (43). The femoral nerve stimulation technique enabled us to examine three key components of peripheral locomotor muscle fatigue: 1) reductions in force output (ΔQ\text{iso}), 2) muscular shortening velocity (CT and MRFD), and 3) prolongation of muscular relaxation time (RT\text{O.5} and MRR). Reductions in the latter two variables are thought to reflect decreases in the maximal rate of weak-to-strong binding and decreases in the

Fig. 4. Myoelectrical activity [integrated EMG (iEMG)], A: mean power frequency (MPF), B of vastus lateralis during environmental normoxic (Normoxia), normobaric hypoxic (Hypoxia), and normobaric hyperoxic (Hyperoxia) trials. Values are normalized to mean of the 1st min. Mean value for iEMG and MPF during each muscle contraction (cycle revolution) was calculated and averaged over each 60-s period. *P < 0.05.

Fig. 5. Group mean data (n = 8) showing relations between percent fall in Q\text{iso} in response to 10-Hz supramaximal femoral nerve stimulation before vs. after exercise and myoelectrical activity [vastus lateralis iEMG (A) and MPF (B)] from the 1st min to the final minute during exercise. The 10-Hz Q\text{iso} data are a representative example and could be replaced by any other stimulation frequency with only minimal differences.

Table 2. Physiological response to the final minute of constant-load exercise

<table>
<thead>
<tr>
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<th>Hypoxia</th>
<th>Normoxia</th>
<th>Hyperoxia</th>
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<tr>
<td>F\text{O}₂</td>
<td>0.15 ± 0.00*‡</td>
<td>0.21 ± 0.00‡</td>
<td>1.0 ± 0.00†‡</td>
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<tr>
<td>C\text{ao₂}, ml O₂/dl blood</td>
<td>18.2 ± 0.6*‡</td>
<td>21.3 ± 0.6†‡</td>
<td>23.8 ± 0.7‡</td>
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<td>Exercise time, s</td>
<td>269.7 ± 21.9</td>
<td>269.7 ± 21.9</td>
<td>269.7 ± 21.9</td>
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<td>Power output, W</td>
<td>313.8 ± 10.2</td>
<td>313.8 ± 12.9</td>
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<tr>
<td>Pedal frequency, rpm</td>
<td>105.7 ± 10.7</td>
<td>105.7 ± 2.5</td>
<td>105.7 ± 2.5</td>
</tr>
<tr>
<td>S\text{PO₂}, %</td>
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<td>95.0 ± 0.6†‡</td>
<td>100*‡</td>
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<td>HR, beats/min</td>
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<td>174.0 ± 3.5†‡</td>
<td>165.1 ± 3.6†‡</td>
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<td>RPE</td>
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<td>6.4 ± 0.4†‡</td>
<td>5.3 ± 0.3*‡</td>
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<tr>
<td></td>
<td>Limb     9.5 ± 0.2*‡</td>
<td>7.0 ± 0.4†‡</td>
<td>6.2 ± 0.4*‡</td>
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<tr>
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<td>f\text{breaths/min}</td>
<td>61.1 ± 5.4*‡</td>
<td>46.0 ± 2.9†‡</td>
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<td>V\text{t}/VC</td>
<td>0.52 ± 0.04*‡</td>
<td>0.56 ± 0.03‡</td>
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<td>V\text{E}, l/min</td>
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<td>V\text{O}, l/min</td>
<td>3.8 ± 0.1* ‡</td>
<td>4.2 ± 0.2† §</td>
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<td>V\text{t}/V\text{O₅}</td>
<td>46.5 ± 1.9* ‡</td>
<td>33.6 ± 1.3* ‡</td>
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<td>Pe\text{T\text{CO₂}}, Torr</td>
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<td>108 ± 1* ‡</td>
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<td>Pe\text{T\text{O₂}}, Torr</td>
<td>30 ± 2* ‡</td>
<td>38 ± 1* ‡</td>
</tr>
</tbody>
</table>

Values are means ± SE for exercise performed at 314 ± 13 W for 4.5 ± 0.4 min. F\text{O₂}, inspired O₂ fraction; S\text{PO₂}, H\text{D₂} saturation; HR, heart rate; RPE, rating of perceived exertion; f\text{t}, respiratory frequency; V\text{t}, tidal volume; VC, vital capacity; V\text{O₂}, O₂ uptake; V\text{E}, minute ventilation; Pe\text{T\text{CO₂}} and Pe\text{T\text{O₂}} instead of PO₂ and P\text{O₂}; *P < 0.05 vs. normoxia; †P < 0.05 vs. hypoxia, ‡P < 0.05 vs. hyperoxia. §Conventional respiratory equations for computing V\text{O₂} from expired gas data cannot be determined by Haldane’s transformation equation at F\text{O₂} = 1.0 (57, 63).
maximal rate of strong-to-weak binding and rate of cross-bridge detachment (46, 53). Reductions in MRR and RT0.5 might arise from slowed Ca2+ sequestering by the sarcoplasmic reticulum and from slowing of cross-bridge detachment rates (2, 21).

Significant alterations from baseline in QO2, MRFD, MRR, and RT0.5 during all conditions confirm that a significant amount of peripheral fatigue was induced in each trial. The significant differences of these changes reflect the beneficial effect of elevated CaO2 from Hypo to Hyper on actin-myosin interaction and/or Ca2+ kinetics and, hence, on fatigue.

**CaO2 Affects Exercise-Induced Peripheral Locomotor Muscle Fatigue**

The new information provided by this investigation was twofold. 1) We revealed highly sensitive effects over a wide range of changing CaO2 on peripheral locomotor muscle fatigue induced by constant-load whole body, high-intensity exercise. 2) This sensitive CaO2 effect on fatigue was shown to be present during the exercise (as revealed by the changed rate of recruitment of quadriceps EMG) and immediately after exercise (as measured by the change in force output in response to supramaximal motor nerve stimulation).

The consequences of a relatively small range in CaO2 on fatigue have been reported by others; nevertheless, controversy remains in the literature regarding this question. Our EMG data agree with those of Taylor et al. (60), who found an exaggerated rate of rise of iEMG during cycling exercise in severe, acute hypoxia vs. normoxia. Our work expands their findings by demonstrating 1) the effects of a wider range of CaO2 and 2) significant CaO2-dependent perturbations of ECC as indicated by differences in pre- vs. postexercise twitch force. Our EMG data do not, however, agree with those of Kaysers et al. (36), who showed no effect of severe chronic hypoxia during constant-load cycling to exhaustion. Our twitch data also do not agree with the results of Sandiford et al. (53), who used electrical nerve stimulation during incremental cycle exercise in severe, acute hypoxia and observed no significant effect on fatigue. However, these authors compared hypoxic and normoxic conditions at significantly different (peak) work rates.

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Our previous study also found a highly sensitive effect of ΔCaO2 on peripheral fatigue (51). We previously reported that the use of small increases in FIO2 to prevent the relatively mild HbO2 desaturation (−7%) and fall in CaO2 (−1 ml/dl) that normally occur during heavy sustained exercise to exhaustion in normoxia resulted in a relatively large (>50%) decrease in QO2 (−33 ± 5 vs. −15 ± 5%, P < 0.05). This effect of ΔCaO2 on force output (ΔQO2) is much greater than that observed in Norm vs. Hyper in the present study, despite increases in CaO2 (−2.5 ml/dl) that were twofold greater than in the previous study. We explain this apparent difference in fatigue sensitivity to ΔCaO2 by differences in exercise duration and the resulting magnitude of peripheral fatigue development. First, given the short 4.5-min duration of exercise in the present study (dictated by the time to exhaustion in Hyper), a relatively small magnitude of peripheral fatigue was evoked during Norm and Hyper (mean of 4 frequencies = −16.5 ± 1.7 and −13.4 ± 2.4%, respectively). Second, on the basis of the present EMG evidence, the beneficial effect of the elevated CaO2 (Norm < Hyper) on fatigue is time dependent (Fig. 4) and could therefore not attain its full potential because of the short duration of exercise and, thus, result in a relatively small, although significant, difference in fatigue between Norm and Hyper.

**Causes of Fatigue via Alteration in CaO2**

Various levels of FIO2 substantially altered CaO2, and O2 delivery, which precipitated a change in VO2 at a given absolute workload and, therefore, a shift in the relative intensity of the exercise, i.e., higher in Hypo and lower in Hyper. We have shown this effect on reducing total VO2 in Hyper (Table 2), and Knight et al. (38) demonstrated this effect specifically on limb VO2 in hyperoxia. It is generally accepted that the percentage of type II fibers activated will increase with increasing relative exercise intensities (44). Additionally, hypoxia per se has been suggested to affect fiber type contribution by attenuating the sensitivity of type III/IV muscle afferents [their stimulation is associated with a preferential recruitment of O2-dependent type I muscle fibers (41)] and, thus, reducing the recruitment of fatigue-resistant type I fibers (20). Consequently, more type II muscle fibers need to be activated under hypoxic conditions to maintain a constant workload. Because type II fibers are associated with an increased rate of metabolite accumulation and fatigue development (see below) compared with type I fibers (22), the O2-dependent change in relative exercise intensity might account for much of the exaggerated peripheral fatigue associated with reduced CaO2. In the present study, the Hypo trial was performed to the limit of tolerance; therefore, the exercise intensity was likely maximal or near maximal. Thus, although compensatory mechanisms might have attenuated the difference in fatigue between Norm and Hyper (both performed at submaximal intensities), such compensation was not feasible during Hyper because of the maximal exercise intensity in this trial.

It is important to consider the relation between muscle O2 supply, muscle bioenergetics, and the rate of metabolite accumulation as potential sources of interference with ECC. The significantly reduced VO2 (~10%) during Hypo compared with Norm indicates that the muscle was likely O2 transport limited (51). An increased muscle metabolic acidosis during heavy exercise is also likely to occur in hypoxia (1), and protons are thought to play a role in causing metabolic fatigue (15, 19). However, recent in vitro studies have questioned the role of H+ concentration in metabolic fatigue (18, 47). Alternatively, the rate of phosphocreatine hydrolysis and concomitant inorganic phosphate (P1) accumulation have been shown to be faster in hypoxia and slower in hyperoxia than in normoxia (35). In turn, it is well known that P1 accumulation plays a major role in the development of fatigue during exercise by impairing Ca2+ release from the sarcoplasmic reticulum (16, 26). Thus, because O2 supply has been shown to influence the accumulation of P1 by its effect on the rate of phosphocreatine hydrolysis, the different rates of P1 aggregation might be the key mechanism explaining the effect of ΔCaO2 on exercise-induced development of peripheral locomotor muscle fatigue.

Finally, it has been established that reducing the work of breathing by about one-half or more during maximal-intensity exercise increases limb vascular conductance and blood flow (33) and reduces peripheral limb fatigue (51). Hypo caused a −26% increase in VE (above Norm), but Hyper had no significant effect on VE during the brief 4.5-min work test. Accord-
ingly, we would expect accompanying increases in the work of breathing to contribute in part to the observed effects of reductions in CaO₂ on peripheral muscle fatigue.

**Task Dependency of CaO₂ Effects**

Mechanisms of peripheral muscle fatigue are “task dependent” (24); thus, the effects of variations in CaO₂ on peripheral muscle fatigue depend on the characteristics of the exercise under study. For example, substantial variations in CaO₂ do not affect Qw under baseline resting conditions (29), fatigue associated with isometric exercise of a isolated muscle group (29), or the fatigue index associated with very brief, predominantly anaerobic, exercise (13). A previous study comparing the levels of fatigue (electrical nerve stimulation) induced by mild-intensity cycling exercise (50% normoxia V̇O₂max) in normoxia and hypoxia for 90 min also failed to demonstrate a significant effect of CaO₂ on peripheral fatigue (52). At these submaximal exercise intensities, compensatory alterations in blood flow and/or O₂ extraction are able to compensate for acute reductions in CaO₂; thus, V̇O₂ is not affected, and fatigue is not exacerbated (12, 38, 61). Thus we caution that the effects of ΔCaO₂ on peripheral muscle fatigue demonstrated in the present study are likely limited to the specific case of whole body, predominantly aerobic, exercise at maximal or near-maximal intensity.

\[ \Delta \text{CaO}_2 \text{ vs. } \Delta \text{PaO}_2 \]

We attribute the effect of increases and decreases in CaO₂ at near-maximum work rate on peripheral fatigue to changes in muscle O₂ transport (CaO₂ × Q, where Q is cardiac output). Although a changing CaO₂ will elicit compensatory responses in Q and limb blood flow, O₂ transport is still significantly altered. The result is that at submaximal work rates the arterial-venous O₂ content difference is altered to maintain muscle V̇O₂, whereas at maximal or near-maximal work rates (as in the present study) O₂ transport and V̇O₂ would be increased in hyperoxia and reduced in hypoxia. These effects at near-maximal work rates have also been shown to occur across the working limb in hyperoxia and hypoxia (38). Presently, we found similar effects, in that total V̇O₂ was reduced at equal work rates in Hypo and Norm. There is also the possibility that changes in PaO₂, per se, independent of ΔCaO₂, might influence muscle capillary and intracellular PO₂, leading to changes in muscle V̇O₂ and peripheral fatigue. However, given the effects of ΔCaO₂ on O₂ transport at maximal and near-maximal exercise (12, 31, 38, 50), the independent effect of PaO₂ would not be required to explain the dependence of muscle function on ΔCaO₂, as presently observed.

**Voluntary Muscle Activation and Central Fatigue**

There was no difference between pre- and postexercise voluntary muscle activation. A near full recovery of voluntary activation after ~3 min following the cessation of exercise has previously been reported (9). Thus, on the basis of the delay between the end of exercise and the start of the fatigue assessment protocol (see above), perhaps the superimposed twitch technique was not adequate to reveal potential central components of fatigue. Additionally, central fatigue is task dependent (28), and the superimposed twitch technique, based on a maximal isometric muscle contraction pre- vs. postexercise, might not be representative of alterations in voluntary muscle activation during dynamic whole body exercise.

The present study was focused only on CaO₂ effects on exercise-induced peripheral muscle fatigue; however, the “central” aspect of fatigue cannot be ignored in this investigation. The trial in Hypo was performed to the limit of exhaustion, and voluntary termination of exercise might have been triggered by central fatigue mechanisms secondary to O₂-sensitive afferent feedback from many sources, including fatigue limb or even a hypoxic brain (28). The Norm and Hyper trials were terminated by the investigators; thus any effect of central fatigue, although potentially present, cannot be revealed by the present design.

In conclusion, the major message of this study concerns the highly sensitive effects over a wide range of increasing and decreasing CaO₂ on peripheral locomotor muscle fatigue induced by constant-load whole body, high-intensity exercise. This sensitive CaO₂ effect on fatigue was shown to be present during the exercise (as revealed by the changed rate of recruitment of quadriceps EMG) and immediately after exercise (as measured by the change in force output in response to supra-maximal motor nerve stimulation).

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